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Master's thesis

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Ichthyosis in the Golden retriever

- Variation of clinical manifestations and breeding aspects in Golden retrievers that are homozygous for the PNPLA-1 mutation

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Preface

This master's thesis was created in the winter of 2013/spring of 2014 at the Division of Genetics and Bioinformatics, Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical Sciences, Copenhagen University.

The aim of this project was to provide veterinarians, breeders and the breeding associations with more knowledge on ichthyosis in the Golden retriever breed, and to be an instrument in the assessment of whether a breeding restriction or breeding recommendation should be introduced.

This current project is the successor to a previous master's thesis done by Emma Bacher. In her study the allele frequency of the alleged causative gene, PNPLA-1, was determined in the Danish population of Golden retrievers.

I would like to say a special thanks to my academic advisor, Professor Merete Fredholm, for her excellent guidance and for having the special ability to always make me walk out her office-door with the sense that I could do this. In addition, my co-advisor dermatology specialist Ph.d Lene Boysen and June Berg from Dyrlæge Boysen should be thanked for their big involvement and encouragement. I would also like to thank all of the participating dog-owners, who took the time to fill out the comprehensive questionnaire, without them this survey wouldn't have been possible.

Further I would like to thank my boyfriend, Rasmus, for loving me, supporting me and being patient with me, when I needed it. I also wish to thank my family; my parents, brother and grandparents for supporting and cheering on me through, not only, the process of writing this master's thesis but also in fulfilling my dream to become a veterinarian.

Finally, I would like to thank to my good friend and co-student Benitta Christensen for using her time to help me improve my text. Golden retriever breeder Kaj Møller from Kennel Grejsdalen should also be thanked for lending me the beautiful picture on the front page.

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Resumé

Ichthyosis er en arvelig hudlidelse, der er demonstreret hos adskillige dyrearter, herunder både mennesker og dyr. I de seneste år er et øget antal golden retrievere blevet diagnosticeret med ichthyosis, der er karakteriseret ved overdreven skællen og hyperpigmentering. Arvegangen er automal recessiv og senest er en mutation i PNPLA-1 genet identificeret som mulig årsag til udvikling af lidelsen.

Der er imidlertid stor variation i graden af symptomer hos hunde, der er homozygote for mutationen. Denne variation er hovedfokus for dette specialeprojekt. Der er ingen kur mod ichthyosis, hvorfor behandling er symptomatisk. Effekten af disse behandlinger vil blive belyst i denne opgave. Ichthyosis' betydning for ejerene blive kortlagt, da dette er ikke tidligere undersøgt. Ligeledes er det relevant at undersøge, om der er baggrund for en avlsrestriktion/avlsanbefaling og hvorvidt denne i så fald kan baseres på genotypning for PNPLA-1 mutationen.

33 ejere af hunde, der er homozygote for mutationen, har besvaret og returneret et omfattende spørgeskema, der efterfølgende er blevet bearbejdet.

Fundende tyder på at en betydelig andel af hundene (25%) er fuldstændig symptomfri og bekræfter samtidigt den anselige variation i symptomer. Kun en lille andel af hundene har svære symptomer. Endvidere har næsten 50% af hundene oplevet bedring i symptomer som følge af de symptomatiske behandlinger.

At have hund med ichthyosis påvirker generelt ejerene minimalt. Som forventet, påvirkes ejere i højere grad jo flere symptomer hunden har. Et flertal af ejerene mener, at man bør reducere forekomsten af ichthyosis gennem avlen, dette ser ud til at være uafhængigt af graden af skæl hos nuværende hund.

På grundlag af fundene i dette projekt konkluderes det, at en avlsrestriktion/avlsanbefaling ikke kan baseres på PNPLA-1 DNA testen. Dette ville medføre, at raske hunde udelukkes fra avl og desuden ville udelukkelse af alle hunde, der er homozygote for mutationen, medføre nedsat genetisk diversitet.

Abstract

Ichthyosis is a skin condition demonstrated in numerous species of animals as well as humans. An increasing number of Golden retrievers have lately been diagnosed with this condition, characterised by generalised excessive scaling and hyperpigmentation. The mode of inheritance is autosomal recessive and a mutation in the PNPLA-1 gene is thought to be causative.

However, the clinical symptoms show great variation among Golden retrievers, which are homozygous for the PNPLA-1 mutation; this variation is the main focus of this project. There is no cure for ichthyosis and subsequently the treatment is based on alleviating symptoms. The effects of such treatments will be evaluated. The implications owners experience from having a dog with ichthyosis is also assessed. In addition an assessment of the need for a breeding restriction/ recommendation and the usefulness of the PNPLA-1 DNA test will be made.

33 owners of Golden retrievers that are homozygous for the mutation participated by answering a questionnaire. The questionnaires were returned and processed.

The findings suggest that a substantial proportion (25%) of Golden retrievers are completely without any signs of ichthyosis and also confirm that among dogs with symptoms, the variation is pronounced. Only a small number of dogs are severely scaling. When evaluating the effects of treatments, improvement in symptoms is seen in almost 50% of the participating dogs.

Owners of Golden retrievers with ichthyosis are generally insignificantly impacted by the ichthyosis of their dogs. And as could be expected, owners are more impacted the more their dogs are scaling. Interestingly, owners generally request a reduction in the incidence of ichthyosis, in spite of the degree of impact this condition causes their dogs.

Based on the findings it is concluded that a breeding restriction/recommendation should not be based on the PNPLA-1 DNA test, since this would exclude healthy animals and because excluding all dogs, which are homozygous for the mutation could lead to loss of genetic diversity.

Contents

1 Introduction

During the years veterinarians have described a generalised excessive scaling condition in Golden retrievers, characterised by pigmented scales, hyperpigmentation and nonpruritic in otherwise healthy dogs (Cadiergues, et al., 2008; Grall, et al., 2012). 'Ichthys' translates to fish in Greek, referring to the fish scale appearance some patients with ichthyosis display (Scott, 1989). The human analogue to these symptoms is called ichthyosis and is a primary disorder of the keratinisation with abnormal differentiation and desquamation of the epidermis (Richard, 2004; Cadiergues, et al., 2008).

Ichthyosis has been recognised in many different species including humans, dogs, cats, alpacas, cattle, rats and llamas with various clinical symptoms, histopathological changes and pathomechanisms (Knox and Lister-Rosenoer, 1978; Belknap and Dunstan, 1990; Credille, et al., 1998; Molteni, et al., 2006; Cadiergues, et al., 2008; Wolff and Johnson, 2009; Scott, et al., 2010).

Lately experimental and investigative studies with emphasis on ichthyosis in the Golden retriever breed have been carried out to elucidate the nature of ichthyosis in the Golden retriever breed (Cadiergues, et al., 2008; Mauldin, et al., 2008; Guaguere, et al., 2009; Grall, et al., 2012). A great phenotypic variation is evident from the clinical studies done so far (Mauldin, et al., 2008; Cadiergues, et al., 2008; Guaguere, et al., 2009). Mauldin and co-workers (2008) speculate that some other factors may influence the degree of lesions.

Ichthyosis in the Golden retriever was first suggested to be an autosomal recessive disorder by Mauldin et al (2008), before being confirmed by others (Cadiergues, et al., 2008; Guaguere, et al., 2009). Grall and colleagues (2012) determined that the causative gene of ichthyosis, an indel mutation in the gene patatin-like phospholipase domain-containing protein 1, PNPLA-1, induces a premature stop-codon and consequently a loss of 74 amino acids. Since this discovery a gene-test for ichthyosis have been introduced on the marked (Antagene, 2014; Laboklin, 2014).

Currently, the Danish Kennel Club has not introduced a breeding restriction on ichthyosis in the Golden retriever, though their ethical recommendations states that dogs with inheritable defects shouldn't contribute to the breeding (Dansk Kennel Klub, 2014). Before a breeding restriction can

be introduced, it is highly relevant to know the full extent of the clinical impacts of ichthyosis on the welfare of the dog, but also the impact this condition exerts on the owners.

1.1 Thesis Statement

The aim of the project is to describe the clinical spectrum of ichthyosis in the Danish Golden retrievers based on replies from owners. Further the effects of symptomatic treatment will be reviewed and a description of how ichthyosis impacts the life of the owners will be made. Moreover, based on the results from the questionnaire along with an analysis of the participating dogs' pedigrees, a conclusion is made about whether reducing the future incidence of ichthyosis via a breeding restriction, based on using the PNPLA-1 DNA test is advisable.

The following questions will be answered using a questionnaire survey and a pedigree analysis.
What are the clinical manifestations of ichthyosis in the Golden retrievers that are homozygous for the PNPLA-1 mutation?
How broad is the span of clinical symptoms among the Golden retrievers, which are homozygous for the mutation?
Does the symptomatic treatments alleviate the symptoms?
To what extent are the owners impacted by their dogs having ichthyosis?
What is the owners' opinion of a reduction of incidence of ichthyosis?
Are some families/lines of Golden retrievers more affected by symptoms than others?
Based on the results, should or could a breeding restriction/recommendation on ichthyosis be introduced?

- And if so, should genotyping for the PNPLA-1 mutation form the basis for a breeding restriction/recommendation?

The first part of this thesis is theoretical and focuses on the current knowledge on the anatomy and function of the skin, followed by a detailed section on ichthyosis and finished with a genetics section, where the genetics of ichthyosis is represented.

The second part of the thesis is the investigative. Here the results from a questionnaire survey among owners of Golden retriever, which are genotyped as being homozygous for the PNPLA-1 mutation, are presented. Alongside this, a pedigree analysis of the participating dogs is performed and the results presented. Finally the results are discussed and concluded upon.

2 The skin

The skin is part of the animals' integument, which translated from Latin means 'to cover'. The integument is the major organ of the body (Monteiro-Riviere, 2006). Working as an anatomic and physiologic barrier against natural wear and invading pathogenic microorganisms is not the only function of the skin. Thermoregulation, water retention, camouflage and communication are among other features of this tissue (Scott, et al., 2001a).

Although there is great difference among animal species and among different, ages and sexes, the skin of all mammals consists of to main portions: The epidermis and the dermis (Scott, et al., 2001a; Monteiro-Riviere, 2006; Miller, et al., 2013a).

2.1 The epidermis

Derived from the embryological ectoderm this outermost part of the skin is characterized by keratinized stratified squamous epithelium (Monteiro-Riviere, 2006). The histology of normal epidermis is depicted in figure 5 on page 16.

The epidermis is constantly undergoing renewal (Miller, et al., 2013a). The keratinocytes move outward from the stratum basale through the different layers of the epidermis, meanwhile undergoing gradual differentiation and maturation before ultimately being sloughed off from the surface of the skin (Scott, et al., 2001a; Dyce, et al., 2002; Miller, et al., 2013a). About 85% of the cells in the epidermis are keratinocytes (Miller, et al., 2013a).

The epidermis is at its thickest dorsal and thin out ventrally on the animal (Scott, et al., 2001a; Monteiro-Riviere, 2006). In areas with dense fur, the epidermis is thinner, since the fur provides increased protection against wear (Miller, et al., 2013a).

The main cellular constituents include keratinocytes, melanocytes, Langerhans' cells and Merkel's cells (Scott, et al., 2001a; Monteiro-Riviere, 2006; Miller, et al., 2013a). Langerhans cells, also called intraepidermal macrophages (Monteiro-Riviere, 2006), are dendritic and mononuclear antigen presenting cells (Miller, et al., 2013a). Merkel's cells or tactile epithelioid cells are connected to adjacent keratinocytes by desmosomes. They are involved in stimulation of keratin growth, can act as slow adapting mechanoreceptors (Monteiro-Riviere, 2006), influence blood flow

and sweat production and are involved controlling the hair cycle. The epidermis is classified into different layers or strata, as seen below (Miller, et al., 2013a).

2.1.1 Stratum basale

The layer residing on the basal lamina on the border of the dermis (Miller, et al., 2013a). This single row of cuboidal or columnar cells has several purposes including anchoring the epidermis on to the basal lamina, mitotic activity of the keratinocytes and communication between the dermis and the epidermis (Kwochka, 1993; Scott, et al., 2001a; Monteiro-Riviere, 2006; Miller, et al., 2013a).

The proliferating keratinocytes are stem cells, which are constantly undergoing mitosis (Scott, et al., 2001a). Production of tonofilament, which is involved in anchoring, as well as development of prekeatin bodies, commences in this layer (Samuelson, 2007). Desmosomes tie the lateral parts of the cells together and hemidesmosomes tie the cells to the basal lamina.

Most of the pigment producing melanocytes resides in this layer (Miller, et al., 2013a).

2.1.2 Stratum spinosum

Depending on the anatomical position, this layer spans from one to two cells thickness in furbearing skin and up to 20 cells in e.g. the footpads. Keratinocytes in this layer derive from the proliferating keratinocytes in the stratum basale (Miller, et al., 2013a).

The morphological appearance is cuboidal or polyhedral (Scott, et al., 2001a). In this layer the cells contain keratin intermediate filaments that creates the cytoskeleton and continues into desmosomes (Kwochka, 1993). The keratinocytes are connected to adjacent cells via the desmosomes, which are found at the end of the cells' spiny processes (Samuelson, 2007). Desmosomes, hemidesmosomes, junctional adhesions and focal adhesions provides the adhesion between layers as well as adhesion between cells in a cell layer (Miller, et al., 2013a).

Desmosomes are primarily found in the epidermis as well as the myocardium (Garrod and Chidgey, 2008; Tomason, et al., 2010). Although the primary function of desmosomes is to provide mechanical strength through adhesion, desmosomes also seem to play a role in cell signalling during e.g. wound healing (Tomason, et al., 2010). These intercellular connections have 3 main parts: a complex of intracellular keratin intermediate filaments, a complex between the intermediate

filaments and adhesion molecules of the desmosome and at last the intercellular connection provided by the desmosomal molecules (Garrod and Chidgey, 2008).

2.1.3 Stratum granulosum

This granular stratum may be absent in some regions of the epidermis, but in areas with a stratum granulosum, the thickness is about one to two squamous cells (Miller, et al., 2013a). Present inside the keratinocytes in this layer are keratohyalin granules containing profilagrin, a filagrin precursor, which is involved in the keratinization process (Monteiro-Riviere, 2006). Lamellar granules containing different kinds of lipids and hydrolytic enzymes are present in small numbers (Scott, et al., 2001a).

The most superficial keratinocytes in this stratum begin undergoing one of the first steps in the keratinization process, a programmed cell death, in which the nucleus and other organelles are degraded (Scott, et al., 2001a).

2.1.4 Stratum lucidum

This transparent homogeneous layer consists of completely keratinized, thin dead cells without nuclei or other organelles (Miller, et al., 2013a). It is absent in most parts of the animal, but is found in hairless areas such as footpads and to some extent in the nasal planum (Scott, et al., 2001a).

2.1.5 Stratum corneum

The most superficial horny layer of the epidermis consists of numerous layers of fully differentiated dead keratinocytes, called corneocytes (Miller, et al., 2013a). In the normal skin these are shed continuously in a controlled rate from the most superficial layer, the stratum disjunction, in a process called desquamation (Kwochka, 1993; Monteiro-Riviere, 2006; Samuelson, 2007).

Cells here are translucent, homogeneous and anuclear and the keratin filaments intracellulary lie parallel with the surface of the skin. The structure of the stratum corneum can be viewed as a brick wall – the corneocytes being the bricks and the extracellular lipid matrix the mortar that links it together (Scott, et al., 2001a).

2.2 Keratinization and scale formation

The keratinocytes have multiple functions including immunity and inflammation responses (Scott, et al., 2001a). In order to maintain the epidermis in homeostasis, the keratinocytes must constantly

undergo three steps: proliferation, differentiation and keratinization in a well-balanced way (Kwochka, 1993). This is a very intricate process involving many different signalling pathways, hormones, enzymes and nutritional factors (Kwochka, 1993; Scott, et al., 2001a).

2.2.1 Proliferation

The proliferation process initiates in the stratum basale from where the keratinocytes move outward and gradually differentiate into the fully developed corneocytes (Scott, et al., 2001a; Dyce, et al., 2002; Miller, et al., 2013a).

2.2.2 Differentiation

As the keratinocytes from the stratum basale move towards the stratum corneum several biological changes takes place in the cell. This is the process of differentiation. The to main processes are keratin synthesis and the aggregation of this as well as the formation of the inner cornified envelop by protein-crosslinking (Kwochka, 1993).

The major function of keratinocytes is the synthesis of keratin, the predominant fibrous protein of the epidermis (Overall, 1997; Scott, et al., 2001a; Dyce, et al., 2002; Miller, et al., 2013a). The term keratin, originates from Greek and can be translated to ‘horn’. Keratin provides the epidermis with the primary barrier towards the environment (Miller, et al., 2013a).

The precursor to keratin, prekeratin is synthesized by keratinocytes in stratum basale and stratum spinosum (Kwochka, 1993; Scott, et al., 2001a; Miller, et al., 2013a). The keratohyalin granules in these layers provide the protein profilagin, which is a precursor for the filament aggregation protein, called filagrin. Filagrin causes the keratin intermediate filaments to bundle together to form parts of the cytoskeleton. In the stratum corneum the filagrin bundles are broken down (Kwochka, 1993).

Formation of the inner part of the cornified envelope starts in the strata spinosum and granulosum, where synthesis of the protein involucrine, keratolinin and loricrine commences. These proteins are cross-linked making a thick cell envelope, which replaces the plasma membrane. A specific type of ceramide, which covers the entire cell and is bound to the inner protein envelope, creates the outer lipid part of the cornified envelope (Kwochka, 1993).

The cell envelope is the structure, which ends up surrounding the corneocyte and functions as protection and structural support. This structure is present in the fully matured corneocytes. Since it is almost impermeable it provides protection against microorganisms and other agents in the environment. The fully differentiated corneocytes contains no phospholipids and hence haven't got a true plasma membrane (Miller, et al., 2013a).

2.2.3 Desquamation

The most important process involved in normal desquamation is synthesis of lipids, but also involves synthesis of protein, the attachment sites of desmosomes, geometry of the cells, mitosis and lytic enzymes. The lipids are found intercellulary as well as in the outer lipid layer of the cornified envelope (Kwochka, 1993).

The contents of the lamellar bodies are released by exocytosis between the stratum granulosum and the stratum corneum and thereby working as a waterproof coating of the cell membrane of the cells in the stratum corneum (Monteiro-Riviere, 2006; Samuelson, 2007).

The corneodesmosomes, which links the corneocytes together, are degraded by proteolytic enzymes resulting in shedding of the corneocytes. To prevent premature death of the keratinocytes, the cells possess additional mechanisms to preserve life (Eckhart, et al., 2013).

Failure to synthesise normal keratin results in faulty differentiation and ultimately in a defective cornified envelope and may cause cutaneous scaling (Kwochka, 1993).

The turnover time, the time it takes a keratinocyte to move from the stratum basale to the stratum corneum, has been investigated using laboratory techniques and is about 22 days (Baker, et al., 1973).

2.2.4 Epidermal lipids

Lipids such as phospholipid, ceramides, free fatty acids and sterols play a key role in the function of the barrier as well as water-retention, cohesion, proliferation, differentiation and desquamation. The type of lipids present in the different layers alternate considerably during differentiation (Miller, et al., 2013a). In the most superficial layers of the epidermis phospholipids and triglycerides are replaced by ceramides, free fatty acids and free sterols (Kwochka, 1993; Scott, et al., 2001a).

Ceramides are the most important lipid involved in the barrier function and the elasticity of the horny layer and hence the polyunsaturated fatty acids are important, because they are components of the ceramides. Linoleic acid is an essential fatty acid in dogs as well as cats and is a constituent of some types of ceramides (Miller, et al., 2013a).

2.3 Pigmentation and hyperpigmentation

Melanocytes are the cells responsible for the pigmentation and thus the different colours of skin and hair (Scott, et al., 2001a; Monteiro-Riviere, 2006; Miller, et al., 2013a). The melanogenesis is the production of melanin (Blood, et al., 2007).

These cells reside primarily in the stratum basale of the epidermis and to some extent also in also in hair follicles, sebaceous and sweat glands (Overall, 1997; Scott, et al., 2001a; Dyce, et al., 2002; Monteiro-Riviere, 2006).

Although there are up to 20 times more keratinocytes than melanocytes, the latter have many important purposes such as camouflage, communication, protection against radiation, activity against free radicals as well as a role in inflammation and immunologic responses (Miller, et al., 2013a).

The embryological origin of melanocytes is the neural crest ectoderm, from were the cells move into the epidermis (Scott, et al., 2001a; Dyce, et al., 2002; Monteiro-Riviere, 2006). Morphologically the melanocytes are characterised by dendrites of varying length, which entwine between the keratinocytes. They have a spherical nucleus and melanosomes, the pigment containing granules (Monteiro-Riviere, 2006; Samuelson, 2007).

The process of melanin production starts in the Golgi apparatus, where the melanosome and its contents are synthesised (Samuelson, 2007). The two main pigment types in the melanosomes are called eumelanines and pheomelanines, which are dark-brown and yellow-reddish pigments, respectively (Scott, et al., 2001a; Monteiro-Riviere, 2006; Miller, et al., 2013a).

The pigments are synthesised from the intermediate dopaquinone through a common pathway (Scott, et al., 2001a). An enzyme called tyrosinase is the catalyst responsible for the conversion of

tyrosine into the end product melanin (Monteiro-Riviere, 2006). Tyrosinase is the rate-limiting factor in melanin production. Whether pheomelanin or eumelanin is produced is determined by the genetic makeup of the animal (Miller, et al., 2013a).

When melanogenesis is completed, the melanosomes move into the dendrites of the melanocyte. Through a series of complex steps, the melanosomes in the dendrites move into the cytoplasm of the keratinocytes and often form a 'cap' structure over the nucleus (Scott, et al., 2001a; Monteiro-Riviere, 2006; Samuelson, 2007).

The final appearance of the skin is determined in turn by the degree of melanisation, the number and size of the melanosomes, the type of melanin produced as well as the distribution of melanosomes (Scott, et al., 2001a; Monteiro-Riviere, 2006). Light also influences the melanin production, this is controlled by the endocrine system (Samuelson, 2007). The absence of stimulation, due to the genetic makeup of the animal, accounts for the constitutive pigmentation, where as the facultative pigmentation is influenced by stimuli e.g. UV light and hormones (Scott, et al., 2001a).

The specific pathomechanism of hyperpigmentation is currently unknown (Scott, et al., 2001a). Hyperpigmentation is caused by an increased melanin-content in the epidermis. Hyperpigmentation may be focal or generalised. The melanin pigments can be deposited in the stratum basale or throughout the epidermis and even in the dermis (Miller, et al., 2013a). Hyperpigmentation may be observed in association with inflammation, neoplasia or hormonal dermatoses (Scott, et al., 2001a).

2.4 The dermis

The dermis, called corium, is the part of the skin beneath the basement membrane (Scott, et al., 2001a; Monteiro-Riviere, 2006; Samuelson, 2007). Unlike the epidermis, the dermis is of mesodermal origin (Scott, et al., 2001a; Monteiro-Riviere, 2006).

The elastic dermis is composed to withstand the wear from movements without changing its shape and accounts for most of the tensile strength of the skin (Scott, et al., 2001a). The main constituent of the dermis is connective tissue, which is irregularly packed and comprised of different kinds of fibers such as elastic, collagen and reticular (Scott, et al., 2001a; Monteiro-Riviere, 2006).

Other structures in the dermis include blood and lymph vessels, nerves, glands, hair follicles and the muscles associated with the hair, the arrector pili muscles (Scott, et al., 2001a; Monteiro-Riviere, 2006). The vessels are responsible for bringing nutrients to the cells of the epidermis (Samuelson, 2007). Fibrocytes, macrophages and mast cells are the predominant cell types in the dermis and the less common cells are plasma cells, fat cells and leukocytes (Monteiro-Riviere, 2006; Samuelson, 2007).

The dermis is occasionally divided into different layers: The papillary layer and the reticular layer (Monteiro-Riviere, 2006; Samuelson, 2007). The first mentioned is the layer just beneath the epidermis and is characterised by its loose connective tissue (Monteiro-Riviere, 2006; Samuelson, 2007). This layer creates flexures, which protrude into the epidermis; this structure is called a dermal papilla (Monteiro-Riviere, 2006; Samuelson, 2007). Within the dermal papilla is a capillary bed, which provides nutrients as well as a way of regulating body temperature (Samuelson, 2007). The reticular layer is thicker than the papillary layer and consists of dense connective tissue. Fewer immune cells are seen here, than in the papillary layer (Monteiro-Riviere, 2006; Samuelson, 2007).

3 Ichthyosis

The term ichthyosis actually covers numerous distinct conditions (Credille and Dunstan, 2008) characterised by a faulty epidermal conformation (Wolff and Johnson, 2009). In the human as well as the dog the condition is grouped into two distinct categories; epidermolytic and nonepidermolytic (Credille and Dunstan, 2008).

Ichthyosis covers a very heterogeneous group of hereditary or acquired conditions with very different causes, clinical manifestations and courses in human medicine (Wolff and Johnson, 2009).

The severity of lesions in humans often decreases with age and the face, hands and feet are usually without involvement. Therapy is symptomatic and consists of hydration of the stratum corneum, keratolytic agents and systemic retinoids (vitamin A analogous) (Wolff and Johnson, 2009).

In veterinary medicine ichthyosis is considered a genodermatosis (Credille and Dunstan, 2008), meaning it is a genetically determined disorder of the skin (Anderson, 2003). The particular type of ichthyosis is characterised and distinguished by the clinical symptoms: the type of scales, the species and/or breed in question, the response to therapy (Credille and Dunstan, 2008) as well as the histopathological abnormalities (Guaguere, et al., 2009).

3.1 Ichthyosis in dogs

In contrast to human medicine, the term ichthyosis in veterinary medicine only covers genetic and/or congenital disorders (Credille and Dunstan, 2008).

Nonepidermolytic ichthyosis is the most frequently observed form of canine ichthyosis (Gross, et al., 2005). Epidermolytic ichthyosis is associated with faulty keratin production and subsequent lysis of the epidermal cells and is characterised by hydropic degeneration of the keratinocytes (Olivry and Mason, 1998; Credille, et al., 2005; Gross, et al., 2005).

Although being a rare condition in the general dog population, some breed tendencies have been identified (Credille and Dunstan, 2008). In breeds such as Norfolk terrier (Credille, et al., 2005), Rhodesian ridgeback, Labrador retriever (Credille and Dunstan, 2008) and possibly in the Cavalier King Charles spaniel epidermolytic ichthyosis with more or less sporadic incidence have been

recognised (Credille and Dunstan, 2008; Hartley, et al., 2012). A splice-site mutation in the Keratin 10 gene is the genetic cause of the mild epidermolytic ichthyosis observed in the Norfolk terriers (Credille, et al., 2005).

The nonepidermolytic form of ichthyosis has been identified in the Jack Russel terrier (Credille, et al., 2009), Cairn terrier, American bulldog, Rottweiler (Credille and Dunstan, 2008) as well as the Golden retriever (Cadiergues, et al., 2008; Mauldin, et al., 2008; Guaguere, et al., 2009; Grall, et al., 2012). Jack Russel terriers have been identified with lamellar ichthyosis, a form of nonepidermolytic ichthyosis, caused by a LINE-1 insertion in the gene coding for Transglutamine 1 (Credille, et al., 2009).

Case reports concerning dogs from other breeds with ichthyosis have anecdotally been documented (Muller, 1976; August, et al., 1988; Scott, 1989; Helman, et al., 1997; Credille, et al., 1998).

3.1.1 Ichthyosis in Golden retrievers

The Golden retriever breed seems to have a high incidence of symptoms of ichthyosis (Cadiergues, et al., 2008; Mauldin, et al., 2008) and express distinct clinical manifestations (Guaguere, et al., 2009; Mauldin, 2013). In 2009 Gauguere et al suggested a genetic cause of ichthyosis due to the high and increasing incidence of clinical symptoms within this breed.

3.1.1.1 Clinical manifestations

The predominant lesions of ichthyosis in Golden retrievers are generalised scaling and hyperpigmentation (Cadiergues, et al., 2008; Mauldin, et al., 2008; Guaguere, et al., 2009; Grall, et al., 2012).

Among the affected dogs significant variation in severity of the symptoms exist. An examination of 40 Golden retrievers diagnosed with ichthyosis showed a considerable variation in the amount of scaling (Guaguere, et al., 2009). The same study found that the type of scale ranges from pityriasiform to psoriasiform to ichthyosiform (Guaguere, et al., 2009). Pityriasiform means small, thin, whitish scales. Psoriasiform is used to describe broader and thicker scales, whereas the term ichthyosiform describes the thick scales, which are hard to detach (Guaguere and Prélaud, 2008). Mauldin et al (2008) also reported large snowflake-like scales.

Figure 1. *Nonepidermolytic ichthyosis in a Golden retriever. Small to large whitish scales.* (Guaguere, et al., 2009)

Figure 2. *Nonepidermolytic ichthyosis in a Golden retriever. Blackish scales over the trunk* (Guaguere, et al., 2009)

Scale size range from 1 to 13 mm and with a variable degree of pigmentation (Cadiergues, et al., 2008; Mauldin, et al., 2008). The colours of the scales range from white to grey and even black (Mauldin, et al., 2008; Cadiergues, et al., 2008; Guaguere, et al., 2009). Different scale size, type and pigmentation are depicted on figures 1 and 2 above.

The scales are distributed symmetrically (see figure 3 below) (Mauldin, et al., 2008; Cadiergues, et al., 2008), and generally footpads and the nasal planum are without involvement (Cadiergues, et al., 2008; Guaguere, et al., 2009). The body regions with the most prominent scaling are the lateral thorax, flanks and ventrally on the abdomen and sternum (Cadiergues, et al., 2008). The minimally affected body areas are ears, face, tail and feet (Cadiergues, et al., 2008; Guaguere, et al., 2009).

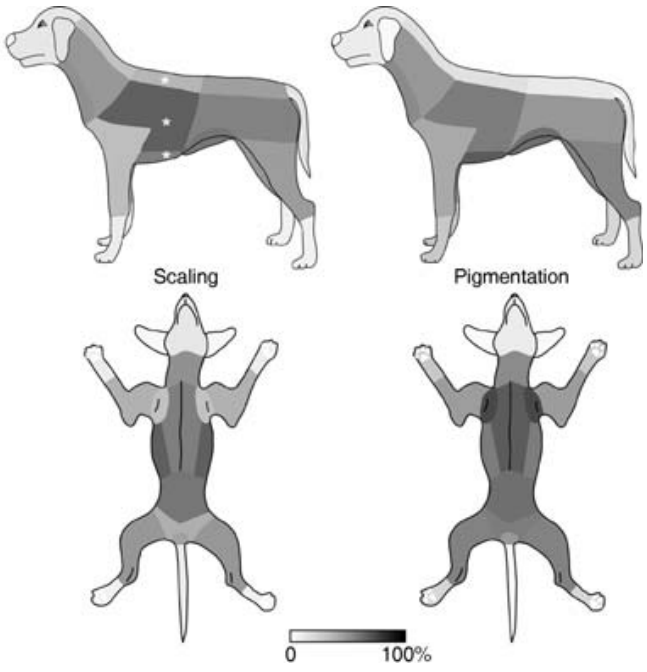

Figure 3:
Distribution of lesions.
Expressed as percentage of maximum score.
Gradation from 0% (white) to 100% (black)

(Cadiergues, et al., 2008)

Hyperpigmentation is primarily ventrally oriented (Mauldin, et al., 2008; Cadiergues, et al., 2008) and is distributed symmetrically (see figure 3 above) (Cadiergues, et al., 2008). The hyperpigmented skin attains a sandpaper-like appearance (see figure 4 below) (Guaguere, et al., 2009). The axilla, sternum, abdomen, lateral thorax, ventral neck, groin and hind limbs are the regions most marked by the hyperpigmentation (Cadiergues, et al., 2008). Hyperpigmentation of the pinnae, tail, face, lumbar and dorsal region is only mild, but may be absent (Cadiergues, et al., 2008). Scaling and hyperpigmentation is correlated in all areas of the body, with the exception of the axillary and inguinal regions. The reason for this may be due to mechanical removal of the scales due to the movements of the dog in these regions (Cadiergues, et al., 2008).

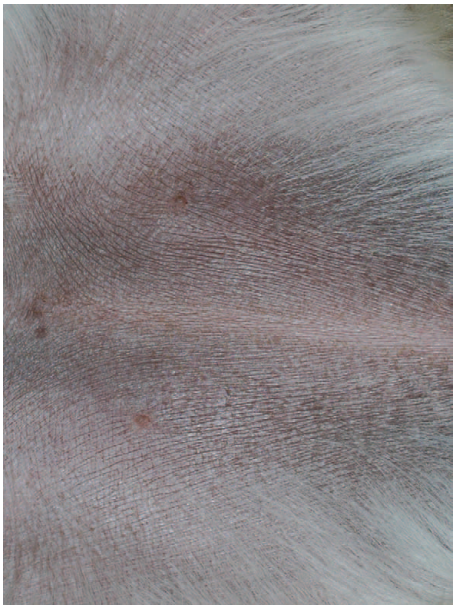

Figure 4. *Hyperpigmentation of the ventral glabrous skin. Note the rough sandpaper-like appearance.*

(Guaguere, et al., 2009)

Ichthyosis in Golden retrievers is rarely pruritic and alopecia is also infrequently observed. Besides the dermatologic abnormalities the dogs seem healthy (Cadiergues, et al., 2008; Guaguere, et al., 2009).

No sex predilection has been demonstrated in the studies on this condition (Cadiergues, et al., 2008; Mauldin, et al., 2008; Guaguere, et al., 2009). While the age of onset of clinical manifestations vary from four weeks (Guaguere, et al., 2009) to 12 years of age (Mauldin, et al., 2008), studies show that the majority of dogs develop symptoms at a young age (Cadiergues, et al., 2008; Mauldin, et al., 2008; Guaguere, et al., 2009).

3.1.1.2 Histopathology and ultrastructural changes

Based on the histopathologic changes ichthyosis in Golden retrievers is classified as nonepidermolytic (Mauldin, et al., 2008; Cadiergues, et al., 2008; Guaguere, et al., 2009). Guaguere and others (2009) also classify this form of ichthyosis as a retention ichthyosis.

Mauldin and colleagues (2008) studied the histopathologic changes in 46 dogs and found laminar orthokeratotic hyperkeratosis with minimal or no epidermal hyperplasia. The stratum corneum is considered to be compact or laminar orthokeratotic (Cadiergues, et al., 2008; Mauldin, et al., 2008; Guaguere, et al., 2009; Grall, et al., 2012). Hyperkeratosis means that the stratum corneum increases in thickness. With orthokeratotic hyperkeratosis the keratinocytes are anuclear (se figure 5 below) (Scott, et al., 2001b).

The hyperkeratotic changes range from mild to moderate (Mauldin, et al., 2008), whereas Guaguere and others (2009) determined the hyperkeratosis as ranging from moderate to severe. The corneocytes were packed more closely than in normal skin (Mauldin, et al., 2008).

Cadiergues and co-workers (2008) detected mild acanthotic changes with a complete or patchy distribution in many of the samples, this finding was confirmed by Guaguere and colleagues (2009) and Grall et al (2012). The definition of acanthosis is thickening of the stratum spinosum. The term is sometimes used interchangeably with hyperplasia (Scott, et al., 2001b).

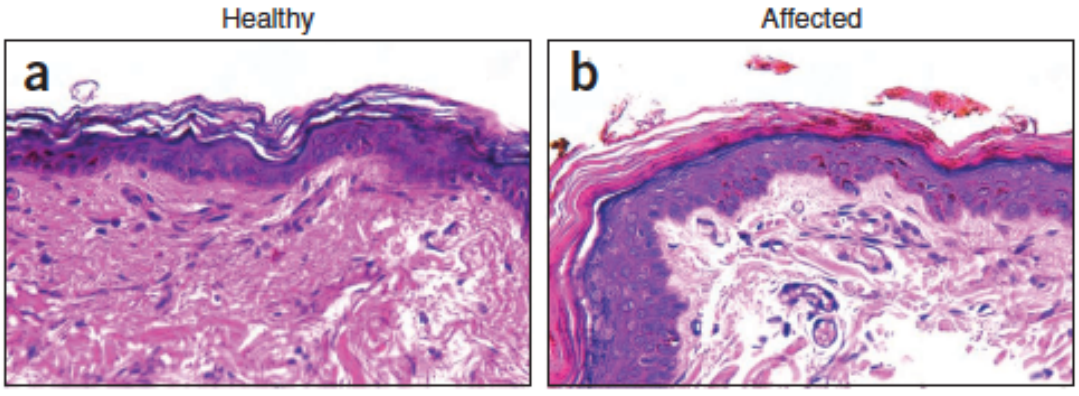

Figure 5. *Hematoxylin and eosin stained skin biopsies for a healthy and an affected Golden retriever. Note the pronounced orthokeratotic hyperkeratosis and acanthosis of the stratum granulosum (b)*
(Grall, et al., 2012)

An increase in unidentified intracellular vacuoles was recognised in the stratum spinosum by Cadiergues and colleagues (2008). Gauguere et al (2009) and Grall et al (2012) also found cytoplasmic vacuoles in the stratum granulosum and subgranular stratum, respectively. Furthermore Grall et al (2012) also found hypergranulosis with increased keratohyalin content, in addition to pronounced desquamation of the outermost loosely packed scales. Hypergranulosis is the term used to describe an increased thickness of stratum granulosum (Scott, et al., 2001a).

The pattern of the hyperpigmentation is patchy, which corresponds with the sand-paper-like appearance of the skin when observed macroscopically (Cadiergues, et al., 2008; Guaguere, et al., 2009).

Cadiergues and colleagues (2008) described hypereosinophilia in the stratum corneum, a result that have not been demonstrated in other studies. Inflammation of the epidermis is rare and if present the changes are mild. Inflammation of the dermis is more often present with a mononuclear or neutrophil infiltrate (Cadiergues, et al., 2008; Guaguere, et al., 2009).

Ultrastructurally numerous convoluted membranes and a crystalline material have been demonstrated (Mauldin, et al., 2008). Another study found what seemed to be remnants of cholesterol crystals and signs of degenerative processes in the intracellular membrane trafficking system (Grall, et al., 2012).

Ultrastructural examination of Golden retrievers with symptoms of ichthyosis showed that the corneocytes remained cohesive or agglutinated and that corneodesmosomes are more numerous than in normal skin (se figure 6 below) (Cadiergues, et al., 2008; Guaguere, et al., 2009). These findings indicate that ichthyosis in Golden retrievers involves an abnormal desquamation process (Cadiergues, et al., 2008; Guaguere, et al., 2009).

Absence of normal degradation and elimination of the melanin is indicated by the presence of melanosomes throughout the epidermis (Guaguere, et al., 2009). No changes in the cornified envelope have been identified (Mauldin, et al., 2008; Guaguere, et al., 2009).

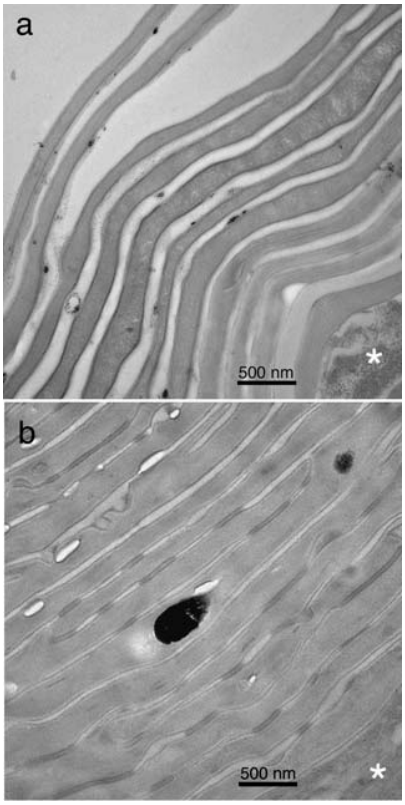

Figure 6. *Transmission electron micrograph of the stratum corneum.*
The stars indicate the most superficial layer of the stratum granulosum.

a) An unaffected dog. The 3-4 most proximal layers of the stratum corneum seem loose and the number of corneodesmosomes is low

b) A Golden retriever with nonepidermolytic ichthyosis. The corneocytes in the stratum corneum are tightly adherent and the number of corneodesmosomes is high.

(Cadiergues, et al., 2008)

Subcutis and adnexae are considered without involvement (Cadiergues, et al., 2008; Mauldin, et al., 2008; Guaguere, et al., 2009).

3.1.1.3 Diagnosis

Previously diagnosing this condition was based on the anamnesis including age of onset and presence of pruritus, a clinical examination to evaluate the symptoms and histopathology in skin-biopsies (Credille and Dunstan, 2008; Mauldin, et al., 2008).

At the present molecular techniques provide the option of genotyping Golden retrievers for the genetic mutation believed to be responsible for ichthyosis. This requires buccal swaps or whole blood in EDTA (Antagene, 2014; Laboklin, 2014).

Depending on the severity of scaling, several different differential diagnoses should be taken into consideration e.g. atopy, parasitic disorders, sebaceous adenitis, hypothyrodism, epitheliotrophic cutaneous lymphoma or metabolic diseases (Mauldin, 2013).

3.1.1.4 Therapy

So far research into corrective treatment of ichthyosis has been very limited, consequently treatment is symptomatic, since ichthyosis is a non-curative condition (Mauldin, 2013).

Recovering of the barrier function of the stratum corneum and a reduction of lesions is currently the aim of therapy, but before therapy is commenced an exact diagnosis must have been made (Mauldin, 2013). It is important the dog is persistently checked for irritations or secondary skin infections, since these dogs have an increased risk of such complications (Guaguere, et al., 2009; Mauldin, 2013; Miller, et al., 2013b).

In trying to manage the condition and alleviate symptoms, topical agents are the primary treatment choice (Mauldin, et al., 2008; Mauldin, 2013). Excessive scaling may be reduced by use of keratolytic agents; this could be sulphur and salicylic acid containing shampoos, which will help break the scales apart (Mauldin, 2013).

Two dogs, a terrier and a Doberman pincher with very severe scaling were among others treated with topical salicyclic acid, along with tretinoin, the therapy alleviated the condition, but was not curative (Muller, 1976). Another case report concerning an American Pitbull terrier, affected by lamellar ichthyosis, showed significant remission, when administered isotretinoin orally (Scott, 1989). The use of retinoid should be minimized because of the relatively high risk of adverse effects (Miller, et al., 2013c).

A moisturizer should be applied to help regain the barrier function and restore water within the skin after showering (Mauldin, et al., 2008; Mauldin, 2013; Miller, et al., 2013b). Use of spot on ceramides and fatty acids, which also should help the barrier function to recover, may extend the intervals between baths (Mauldin, 2013). Scott (1989) used a lactic acid (Humilac) containing rinse on one dog with a resulting significant improvement in the degree of lesions.

Although corticosteroids can be used temporarily to decrease the degree of scale formation, the negative effect on the barrier function could make it contraindicated (Mauldin, 2013). Supplements of oral fatty acids could also be helpful (Guaguere, et al., 2009; Mauldin, 2013).

In a study 8 dogs had been treated with some of the abovementioned treatment options without persistent improvement based on the owners' responses (Cadiergues, et al., 2008).

The result of treatment is dependent on the compliance of the owner (Mauldin, 2013). To follow through with the treatments may prove too laborious or expensive for owners (Scott, 1989).

3.1.1.5 Prognosis

Even though the Golden retrievers affected with ichthyosis generally display mild symptoms, the condition may cause a predisposition to secondary pyoderma (Mauldin, et al., 2008).

Based on the owners description Cadiergues and co-workers (2008) found a great difference in the progression of symptoms. In one group of dogs the severity of symptoms were unchanged and in the other group of dogs various specific factors that worsen the symptoms were identified e.g. season, whelping and moulting (Cadiergues, et al., 2008).

4 Genetics

4.1 Genetics of the dog

Vilá et al (1997) demonstrated that the wolf is the common ancestor of all dogs. Since taming of the wolf targeted breeding have created extremely diverse morphological appearances and behavioural traits such as herding, guarding, hunting and companionship (Vilá, et al., 1997).

The dog is now a separate species from the wolf and an extensive amount of work have lately been directed at the study of the canine genome. One reason for this is because of the unique population genetics. Many breeds of dogs have a very low number of founders making them an isolated gene pool (Sutter and Ostrander, 2004). This fact makes dogs a fitting model for some human genetic diseases, as seen in the nonepidermolytic ichthyosis of Golden retriever and the autosomal recessive congenital ichthyosis of humans (Grall, et al., 2012). The dog genome holds 19.000 genes (Ostrander and Wayne, 2005) distributed on 38 pairs of chromosomes and two additional sex chromosomes, X and Y (Sutter and Ostrander, 2004).

4.2 Genome wide association study

The goal of a genome wide association study (GWAS) is to identify an association between a specific phenotype and a specific genomic region. The association is a statistical observation; individuals with a condition in common also have e.g. an allele in common (Strachan and Read, 2011a).

Single-nucleotide polymorphisms, SNPs, are used as genetic markers to detect the common genetic variation among affected individuals (Pearson and Manolio, 2008). A SNP is the simplest form of genetic variation between individuals. It consists of a substitution of just one nucleotide for another nucleotide. In humans it is estimated that a SNP is present for every 1000 basepairs. These genetic changes are considered stable in the genome (Shastry, 2009). SNPs are more numerous and less mutable than microsatellites and are therefor the genetic marker of choice in GWAS (Strachan and Read, 2011a).

GWA studies utilize a phenomenon called linkage-disequilibrium; LD. LD describes a genetic relationship between specific combinations of alleles that occur more often (or less often), than would be expected based on individual allele frequencies (Pearson and Manolio, 2008; Strachan

and Read, 2011a). SNPs with a high degree of LD are often inherited together (Pearson and Manolio, 2008). The proportion of variation of one SNP being explained by the other SNP is calculated statistically and ranges from no association (0) to perfect correlation (1). Using statistical programmes the significance of the association between the phenotypic trait and alleles can be determined. Visual plots of the calculated P-values are made on a logarithmic scale on the y-scale and genomic location on the x scale. Plots with a value of more than 7 are considered associated with the trait in question (Pearson and Manolio, 2008).

GWA studies can be designed in different ways. A case-control design is the most frequently used. Here allele frequencies of cases (individuals with a specific trait) are compared with the allele frequencies of controls (individuals that are without the specific trait). Other designs include trio and cohort GWA studies (Pearson and Manolio, 2008).

The precision with which the cases and controls is found are essential for reducing the risk of bias in the case control study. The cases must be diagnosed precisely and correctly and since many cases are sampled from clinical sources this group may not include mild or fatal cases. The controls enrolled in the study should originate from the same population as the cases and should go through extensive diagnosing to ensure a disease-free status. Misclassification of individuals in the groups investigated will lead to loss of power of study and may lead the results toward no association (Pearson and Manolio, 2008).

The GWA study design can determine a genomic region, a candidate gene, that is associated with a specific trait, but further information regarding the gene function is often not defined (Pearson and Manolio, 2008). Hence, when a region of interest has been identified using a GWAS, a candidate gene approach can be initiated as was done in the quest to find the causative gene of ichthyosis in the Golden retriever (Grall, et al., 2012).

A candidate gene is a gene believed to be involved in the expression of the phenotype under investigation, due to the already known function of this gene (Pearson and Manolio, 2008).

4.3 Genetics of ichthyosis

Mauldin and co-workers (2008), who did one of the first in depth studies regarding ichthyosis in Golden retrievers, suggested an autosomal recessive mode of inheritance. This assumption was based on the fact that both genders where equally affected and that symptomatic dogs had asymptomatic parents (Mauldin, et al., 2008). Cadiergues and colleagues (2008) also did a pedigree analysis and too suggested a hereditary component due to the clustering of affected relatives. They also suggested that ichthyosis was an autosomal trait. However an oligogenic or autosomal dominant with incomplete penetrance mode of inheritance was not precluded (Cadiergues, et al., 2008). Later work by Gauguere and others (2009) also determined an autosomal recessive mode of inheritance.

As mentioned previously various breed-specific ichthyoses have been recognised clinically. In both the Norfolk terrier and the Jack Russell terrier, however, the genetic and molecular causes of the conditions have been identified, since the individually implicated genes were previously identified in humans (Credille, et al., 2005; Credille, et al., 2009). This genetic correlation between canine and human ichthyosis was also exploited in a study by Grall et al (2012). They used Golden retrievers affected by ichthyosis as a model for a relatively similar human autosomal recessive congenital ichthyosis.

When performing a GWAS Grall et al (2012) found a homozygous region from 8.55 to 9,66 Mb on canine chromosome 12, which was demonstrated in all 20 affected Golden retrievers, but in none of the 20 control dogs from the same breed. This 1,1 Mb area of interest contains 21 annoted genes, including the relatively uncharacterized patatin-like phospholipase domain-containing protein 1, called PNPLA-1. This gene was considered a suitable candidate gene for further studying, because of the PNPLA family's involvement suggested involvement in triglyceride lipolytic and/or lipogenic activity.

The PNPLA-1 or patatin-like phospholipase domain-containing protein 1 is a member of the PNLA family (Kienesberger, et al., 2009). The family consists of PNPLA-1 through PNPLA-5. Besides PNPLA-1 all members have been assigned diseases and/or functions. Although PNPLA-1 is a member of a group of lipid hydrolases the exact function is so far merely presumed. PNPLA-1 showed no triglyceride lipase activity, which is the case for PNPLA-2. The PNPLA-1 protein is

supposed to be associated with the lipid organisation in addition to the homeostasis of the epidermal barrier (Grall, et al., 2012).

A mutation screening on the candidate gene PNPLA-1 was carried out on 12 affected and 12 unaffected dogs. PNPLA-1 was considered a suitable candidate gene by Grall and others (2012), since the neighbouring gene is implicated in a human variant of ichthyosis and because the function and the product of the gene had never been described previously.

Sequencing of the mutated PNPLA-1 gene demonstrated an insertion-deletion (indel) mutation. This indel contains a 3 base pair deletion followed by an insertion of 8 base pairs in exon 8, which resulting in an insertion of 5 base pairs. This mutation causes a reading frame shift and subsequent expression of a premature stop codon. The consequence is a loss of 74 amino acids in the C-terminal ending (Grall, et al., 2012). Figure 7 shows the graphical structure of the PNPLA- 1 gene and the structure of the wild-type and mutated PNPLA-1 protein.

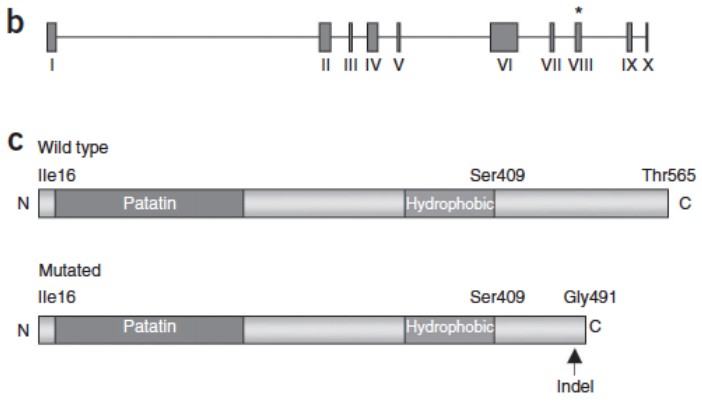

Figure 7.
b) *The structure of the PNPLA-1 gene. The * indicates exon 8, where the indel have been demonstrated.*

c) *The predicted structure on the wild-type and mutated PNPLA-1 protein, respectively.*

(Grall, et al., 2012)

Real-time polymerase chain reaction analysis was performed to determine the expression of PNPLA-1 in the skin of 23 dogs. In accordance with the clinical findings in Golden retrievers with ichthyosis, Grall et al (2012) observed strong expression of the PNPLA-1 gene in the skin, predominantly in the keratinocytes and no activity in the fibroblast. Since the mRNA from the mutated PNPLA-1 is expressed at a considerably lower level in the affected dogs than in healthy and carrier dogs, it was hypothesised that the mutation causes a non-functional protein (Grall, et al., 2012).

In vitro culturing of keratinocytes showed no expression of PNPLA-1 in the earliest stages of differentiation in the normal keratinocytes, but increases during differentiation. In the mutated keratinocytes no PNPLA-1 was detected. When studying the differentiation capacity, it was shown that the earliest phases of differentiation are independent of PNPLA-1 (Grall, et al., 2012).

A defective transport of lipid via the lamellar bodies may be excluded as the pathological cause of symptoms, because the PNPLA-1 protein is not localized to the lamellar bodies, but instead to the keratin filaments in the cytoplasm. Speculations are made on PNPLA-1 activity possibly being involved with the cytoskeleton. Other findings in the same study suggest that lack of PNPLA-1 protein affects the later stages of keratinocyte differentiation, lamellar body function and formation (Grall, et al., 2012).

The ultrastructural findings of abnormal membranes and vesicular material in the granular layer suggest the mutated PNPLA-1 protein causes defects in lipids and consequently causes changes in the membrane integrity, in the membrane trafficking and perhaps in the endocytic pathway (Grall, et al., 2012).

The laboratory Antagene in France estimates that 50% of the European Golden retriever population is carrying the mutation (Antagene, 2014). Bacher (2012) did a survey on the allele frequency in the Danish Golden retriever population. She found that 48,2% of the studied Golden retrievers were homozygous for the mutated allele, 42,5% were heterozygous and the resulting 9,4% were homozygous for the wild-type allele. However, these findings may be overestimated (Bacher, 2012). A Swiss study found a distribution of genotypes as follows: 32% were homozygous for the mutated allele, 49% were heterozygous and 20% were homozygous for the wild-type(Lipska-Owszarek, et al., 2011).

5 Method

5.1 Questionnaire

A total of 36 Golden retriever dogs were recruited in the study. All dogs enrolled had been genotyped before commencing this study commenced and were found homozygous for the PNPLA-1 mutation. A questionnaire (see appendix 1), created by June Berg and Lene Boysen, was sent to the owners of all Golden retrievers homozygous for the mutation. The dogs had previously participated in a master's thesis project or were located based on their ichthyosis status on Hundeweb, the Danish kennel club's complete database (Dansk Kennel Klub, 2014; Bacher, 2012).

Reminders were sent and phone calls made to owners, who hadn't returned the questionnaire, to ensure the highest number of participants possible.

5 dogs had the same owner, as were the case for two other dogs. Therefore the parts of the questionnaires regarding the significance of ichthyosis to the owner are reduced to one answer from each owner. The answers regarding symptoms and the condition of the specific dog were used individually.

The participating dogs have each been assigned a letter or a letter combination due to the anonymity of the responses. All answers and the associated letters from the questionnaire were typed into Excel and is presented in appendix 2. Dogs with yellow markings in appendix 2 are disregarded from the survey because of allergies. Dogs **AA, BB, CC, DD** and **EE** have the same owner**.** Dog **S** and **T** have the same owner.

Where scales from 0 to 10 were used (question 11, 14, 15, 27 and 30) to rate the different aspects, the scale have afterwards been measured and assigned into three categories of equal length; low, moderate and high or none/mild, moderate and severe/very severe.

To show the distribution of scales and hyperpigmentation of the Golden retrievers with these symptoms, the figures from question 7 and 13 have been divided into different body regions, as seen in appendix 3. The distribution proportions indicated on the figures are based on an educated estimate of the shading done by the owners.

The questionnaire lacked a question, where owners could indicate the gender of the dog. The genders were instead recovered from the pedigrees in the Danish Kennel Club's database (Dansk Kennel Klub, 2014) and assigned to each dog in appendix 2.

In addition to the question in the questionnaire one or two questions were emailed to the owners. The question to all owners regarded how many litters of Golden retrievers they have had. The second extra question was sent to owners, who had partially or completely agreed to the statement that the incidence of ichthyosis should be reduced trough breeding, since it is an inherited condition. The questions, response options and answers are seen in appendix 4.

A substantial proportion of the questionnaires had been faulty filled out and mistakes to different extents was present throughout the questionnaire, but due to the low number of participants, it was not reasonable to exclude all of these questionnaires. Some owners didn't respond to all of the questions they were intended to, hence the number of participating dogs/owners in each question will vary. The percentages presented herein are relative to the number of answers in the given question, not to the total number of participating dogs. Where questions have been answered incorrectly, the particular answer has been overlooked.

In the second part of question 27, the owners were asked to indicate the degree of agreement with various statements. For simplicity reasons, the answer options 'completely' and 'partially' agree have been combined to 'agree', and the same have been done with the answer options 'completely' and 'partially' disagree.

Due to the design of the questionnaire, there are multiple questions, where the owner should answer questions regarding the presence of scales and pigmentation. Therefore determining the presence of these symptoms requires some degree of interpretation of answers. Current or previous presence of scaling and hyperpigmentation were based on question 1, 2, 6 and 12. Further, some owners don't respond to question 1, 2, 6 and/or 12, which is interpreted as absence of symptoms, but later answer other questions regarding scaling and hyperpigmentation. These answers are disregarded with respects to presence of scales etc.

Due to the large quantity of questions and answers only questions with substantial and/or interesting results are being presented in this thesis.

5.2 Pedigree analysis

The 4 generation pedigrees of the participating dogs were retrieved from the Danish kennel Clubs' database, Hundeweb (Dansk Kennel Klub, 2014). For some of the dogs a complete 4 generation pedigree were not available. The inbreeding coefficients are presented on each pedigree based on 3 generations. The inbreeding coefficients are seen in appendix 1.

The dogs were divided into groups, one group of dogs presented with the greatest degree of scaling (question 11) and one group with the least degree of scaling. The kinship among the individuals in each group was described based on their individual pedigrees as well as the kinship of dogs between the groups. The kinship among dogs is described on the basis of parents and grandparents.

6 Results

6.1 The questionnaire

36 questionnaires were filled out and returned by the owners. Three questionnaires were disregarded since these dogs suffered from various allergies, making it impossible to distinguish between symptoms of allergy and ichthyosis, and because the owners' perceptions of the impact of ichthyosis may have been influenced by the allergies.

6.1.1 Clinical manifestations

Of the 33 dogs that were included, 18 were female and 15 males. 15% of the participating dogs are asymptomatic females and 9% are asymptomatic males, 5 and 3 dogs, respectively. Of the dogs with symptoms 13 are females and 12 were males, corresponding to 40% and 36%, respectively.

8 dogs, corresponding to 25% of the dogs, are considered by the owners to be without scaling, hyperpigmentation, dry skin or other symptoms of ichthyosis. 69% of the Golden retrievers display scaling. 6% of the dogs were seemingly hyperpigmented, but without scaling. A total of 16 dogs or 50% of the dogs showed both scaling and hyperpigmentation.

Among the scaling dogs 20% are classified with severe/very severe scaling. 35% were classified with no or mild scaling and the remaining 45% with moderate scaling. 25% of owners indicated no or mild hyperpigmentation, moderate hyperpigmentation in 47% of cases and 21% of dogs showed severe/very severe hyperpigmentation.

In question 6 the owners are requested to answer whether their dog presently or previously have displayed abnormal scale formation. 24% of the 69% of owners, who had previously confirmed the presence of scales in question 1 and/or 2, respond no to this question.

The colour of the scales ranged from white to grey and black.

Undiagnosed pruritus was present in 9% or 3 cases and in one additional case the pruritus was diagnosed as being a symptom of ichthyosis by a veterinarian.

The distribution of scales is shown on appendix 3. The area where most dogs display scaling is the lateral trunk. 90-95% of the dogs with scales show this on the most ventral part of the trunk. The dorsal part of the trunk was scaling in 30% of cases. In 50% of the dogs scaling was indicated on the sternum. 15-20% of the dogs displayed scaling on the proximal parts of limbs. No owners indicated scaling in the head region, distal limbs or tail region.

94% of 16 dogs displayed hyperpigmentation in the ventral trunk (see appendix 3). Hyperpigmentation was indicated on the sternum by 44% of owners. All other body regions were without involvement in these dogs.

In question 15 owners are asked to consider the impact of ichthyosis on their dogs. Overall 94% of the owners indicate that the impact of ichthyosis is none/mild and the remaining 6% find the impact moderate. No owners indicate severe/very severe. For all the asymptomatic dogs the impact is classified as none or mild.

65% of the scaling dogs with a known age of onset were 2 months old or less. In 20% of cases the age of onset of scaling were older than two months and younger than one year. This means that 85% of the dogs, which developed scales, did so within the first year of life. One dog developed scales at 8 years of age. The hyperpigmentation commenced at age 6 month or less in 50% of the cases.

In 50% of cases the owners assess that the symptoms of ichthyosis has been reduced since these commenced. In addition 45% claims that the level of symptoms are stable. The symptoms seem to have exacerbated in 5% (one dog). 36% of 22 dogs that at some point showed clinical symptoms of ichthyosis have previously had or is having one or more symptom-free period.

6.1.2 Treatments

The owners were asked about the effect of various symptomatic treatments, which are usually recommended by veterinarians. The following percentages are calculated based only on the dogs displaying symptoms of ichthyosis.

The owners were asked to rate the effect of changing the feed, if this had been attempted. 88% of the dogs had been subjected to change of feed. 38% of these dogs had experienced good or some effect from changing the feed. The remainder were without effect.

Another question concerned the effect of supplementation with fish-oils and the period of use. 70% of the dogs had been given these supplements; a corresponding 30% of the dogs with symptoms of ichthyosis had never been given this. For 3 dogs the effect was unknown, since these dogs had always been supplemented. 31% of the dogs experienced some or good effect from addition of fish-oils according to the owners and the rest of the dogs gained no effect from the fatty acids.

The owners were requested to indicate the period, they had been supplementing with fish oils. 17% of owners had given their dogs supplements for two months or less and a resulting 83% had been supplementing for more than two months.

The last treatment-question concerned showering of the dog. A total of 23 owners of dogs with symptoms of ichthyosis responded to these questions. 65% of the dogs were showered in only shampoo. Shampoo and conditioner were used on 22% of the dogs. For the remaining 3 dogs (13%) nothing was used when showered. 70% of the dogs showed no effect, the remaining 30% of dog displayed some or good effect.

Considering only the cases where shampoo and conditioner were used, 80% of the dogs showed some or good effect. 33% of the dogs were showered at least once a month. In 73% of cases showering was done more than once every 3 months. 27% of the dogs were showered less than four times a year. Keratolytic shampoos seemed to be used by three owners.

When totalling the dogs with some or good effects from one or more of the three-abovementioned treatment options (feed change, fatty acid supplementation and showering), 46% of the dogs experienced positive effects.

6.1.3 Significance of ichthyosis

In this part of the questionnaire the questions are concerned with the owners perception of various aspects of having a Golden retriever with ichthyosis and the personal implications that accompany having a dog with this condition.

In the first part of question 27 the owner is requested to rate the significance of being the owner of a Golden retriever with ichthyosis. 77% of all owners reply no/little importance, 15% answer moderate and 8% reply great/very great importance. All 7 owners of asymptomatic dogs respond no/little importance. 68% of owners of dogs with symptoms answer no/little importance, whereas 21% answers moderate and the remaining 11% respond great/very great importance.

80% of the owners, who rated that the significance of having a Golden retriever with ichthyosis as moderate or great/very great also rated the degree of scaling (question 11) to be severe or very severe.

83% of owners who responded moderate or great/very great significance in the first part of question 27 also agreed that the incidence of ichthyosis should be reduced through breeding. The remaining 17% disagreed to the statement concerning a reduction of ichthyosis. A total of 20 owners responded no or little importance to this part of question 27. 70% of these agreed with the reduction statement and the remainder, 30% disagreed.

In the second part of question 27 the owner is asked to indicate the degree of agreement with various statements.

One statement concerned whether the incidence of ichthyosis should be reduced through the breeding programme, since this is an inherited condition. In the group of asymptomatic dogs 57% of the owners agree and the remaining 43% oppose. 80% of the owners of dogs with symptoms agree with a reduction and the corresponding 20% disagree. In total 74% agree and 26% disagree.

29% of the owners, who agree with a reduction in the incidence of ichthyosis, have indicated severe/very severe degree of scaling. 42% of owners indicated moderate and the remaining 29% rated the degree of scaling as being none/mild. The degree of scaling among dogs of owners, who disagreed with the statement, was equally divided between none/mild and moderate.

In question 29 the owner is requested to indicate the degree of importance of a future golden retriever having ichthyosis. The response options have been grouped into no/little importance and

great/very great importance. In the asymptomatic group of dogs 86% indicates no/little importance and the remaining 14% answer great/very great importance. For the dogs with symptoms 72% respond no/little importance and the corresponding 28% replies great/very great importance.

To 77% of owners of mildly or moderately scaling dogs, the ichthyosis status of a new dog is of little importance. Thus, the remainder, 23%, considers it of great/very great importance. For 75% of owners of dogs with severe scaling, it is of great importance whether a new Golden retriever has ichthyosis, while 25% (one owner) responds little importance.

35% of owners, who agree to a reduction of the incidence of ichthyosis through breeding, indicate that it is of great/very great importance whether a new Golden retriever has ichthyosis. 65%, who agree to a reduction, respond no/little importance. Of the previously mentioned 35%, 83% have rated their dog as being moderately or severely/very severely scaling. To all owners, who disagree with the reduction of ichthyosis statement, it is of no/little importance whether a new dog has ichthyosis.

6.2 Extra questions

13 responses to the second extra question were received (see appendix 4). This question regarded the reason why owners agree that the incidence of ichthyosis should be reduced through breeding. 62% of these owners believe that the incidence of all known inherited conditions should be reduced, regardless of the degree of implications the condition might cause. 15% believes the clinical implications the condition cause their dog is too extensive, answer option A. 8% of owners mostly agreed, that the reputation of the Golden retriever breed was the reason to reduce the incidence, answer option C. The remaining 15% answered option B, which is concerned with the implications of having a dog with ichthyosis is too great.

When asked which option the owners agreed with the least, 54% of the owners replied option A, the clinical implications of the condition is too extensive for the dog. 23% of owners responded B, the implications of having a dog with ichthyosis are too great for the owner. 15% least agreed with D, the incidence of all inherited conditions should be reduced. None of the owners responded C.

6.3 Pedigree analysis

6.3.1 Inbreeding coefficients

Three dogs, corresponding to 9% of the dogs had inbreeding coefficients of more than 0%. The coefficients were, 12,5%, 9,375% and 3,125%.

6.3.2 Kinship

The dogs were assigned into two groups. The letters assigned to each individual dog in appendix 2 are used instead of their names for simplicity. The first group consists of the 4 most severely scaling dogs, based on question 11. The other group consists of the 8 asymptomatic dogs.

The dogs belonging to the group of scaling dogs are D, J, O and Z. These dogs have no parents or grandparents in common.

The dogs belonging to the asymptomatic group are F, M, N, P, S, T, Æ and Å. The kinship among the dogs in this group is as follows:

- S and T are mother and daughter. And S is grandmother to P
- T and Å have the same father
- P and S are grandchild and fathers mother
- P, T and Å have the same fathers father
- P and Å the same mothers father

The kinship between the two groups is as follows:

- The fathers father of dog D is the father of dog Æ

The kinship among all the dogs is as follows:

- A, N and W have no ancestors in common
- H and M are littermates
- C, U and Q are littermates and have no ancestors in common with other dogs
- CC and V are littermates
- M, CC, V, H have the same father and this father is also grandfather to F and BB
- CC, V and DD have the same mother and this mother is also the grandmother of F and H

- L, CC, V, DD, K and AA have a grandmother in common
- AA is the mother of H
- DD and AA are littermates and their father is the grandfather of M and H
- F and J have the same grandfather
- J and L share a grandmother
- Z and X share a grandfather
- O and Ø have their father in common
- S is Y's grandmother
- G and Y have the same father
- K and L have the same mother
- BB and EE have the same father
- R's father is the grandfather of EE and BB
- I's father I R's grandfather
- K's father is grandfather to E

7 Discussion

Before commencing this study one could have expected that all participating dogs would display symptoms of ichthyosis to some degree, since Grall and colleagues (2012) genotyped 320 dogs for the PNPLA-1 mutation and found that all affected dogs were homozygous for the mutation and unaffected was found be either heterozygous or homozygous for the wild-type. However, in the present study only 69% of dogs are scaling (with or without hyperpigmentation), and an additional 6% exhibit only hyperpigmentation.

Although taking misclassification of dogs into account, it is remarkable that dogs, which are homozygous for the alleged causative mutation can differ to such an extent in phenotype.
This finding is in contrast to the study done by Grall et al (2012), where all dogs homozygous for the mutation, displayed some symptoms of ichthyosis.

That 25% of dogs are completely free of symptoms, may be overestimated since one could suspect various reasons why the owner would deny that their dog is showing symptoms. One reason could be that these dogs actually have mild symptoms, which are not considered 'abnormal' by the owner. This thought could also be the cause of the contradiction in question 6, where almost 25% of owners, who previously have confirmed the presence of scaling, negate the presence of 'abnormal' scaling. Another reason to reject the presence of symptoms may be that owners do not think of ichthyosis as a problem and therefore might be more prone to minimize the degree of symptoms. The dermatology specialist Lene Boysen have seen some of the asymptomatic dogs and have confirmed absence of symptoms (Boysen, 2014), thus confirming that some of these dogs are actually without symptoms.

The age of onset of clinical manifestations was established in this current survey to be similar to what has previously been demonstrated (Cadiergues, et al., 2008; Mauldin, et al., 2008; Guaguere, et al., 2009). The majority of dogs develop symptoms at a very young age. 65% of dogs showed symptoms of ichthyosis before 2 months of age. The symptoms were often recognised when the puppies were vaccinated the first time (about 8 weeks of age), hence symptoms may be present before 2 months of age. As Mauldin and co-workers (2008) also predicted, it could be difficult for the owners to determine the age of onset, since mild scaling may be undetected for a period.

The white to black colour scheme of the scales is in agreement with that demonstrated in previous studies (Cadiergues, et al., 2008; Mauldin, et al., 2008; Guaguere, et al., 2009).

Though the distribution-figures of scaling and hyperpigmentation are not designed in the same way in the questionnaire and by Cadiergues and co-workers (2008), they are comparable. First, the distribution of scales and hyperpigmentation is symmetrical (Cadiergues, et al., 2008; Mauldin, et al., 2008), this is also indicated by the owners in the present study. Second, the areas with a high degree of scaling in the literature is the lateral trunk, sternum and ventral abdomen (Cadiergues, et al., 2008), which are also the areas most affected in the dogs in this study. In addition, head, tail and distal limbs are unaffected in this study as well as in earlier studies (Cadiergues, et al., 2008; Mauldin, et al., 2008). Hyperpigmentation is generally distributed ventrally (Cadiergues, et al., 2008), which is also the case in 95% of the dogs in the current study.

A relatively mild nature of ichthyosis in Golden retrievers is illustrated by the responses to the degree of scaling and hyperpigmentation displayed by the dogs. Only one in five dogs display scaling categorised as severe/very severe and the same is the case for the dogs with hyperpigmentation. This notion is further supported by the fact that 94% of owners classify the impact of ichthyosis on their dog as being none/mild and that very few dogs show pruritus that have been or possibly could be related to the ichthyosis. Further, the condition seemed stable in almost half of the dogs and moreover a substantial number of dogs experienced partial remission, periods without symptoms and even complete remission.

Making the owners rate the degree of scaling and hyperpigmentation in their dogs can prove problematic, since the owners most likely doesn't have the necessary qualifications or other cases of ichthyosis to compare to. In addition, owners' tolerance and sensitivity towards the scaling and hyperpigmentation may be very different.

Despite ichthyosis in the Golden retriever breed in general showing a relatively mild course of clinical symptoms, compared to that of other dog breeds, the impact of ichthyosis in some Golden retrievers and on the owners should not be disregarded. Even though none of the owners classified the impact of ichthyosis on their dog to be severe, some cases of ichthyosis in Golden retrievers can be severe with high amount of scaling and pruritus as seen in this study in four dogs and at least one

dog, respectively. As well as the impact on the dogs, some owners become apprehensive and worried and may struggle with the, at times, overwhelming amounts of scaling.

Ichthyosis is a non-curative condition and the effects of symptomatic treatments are continuously debated (Muller, 1976; Scott, 1989; Mauldin, 2013). Although some older studies seem to be demonstrating effects from using shampoos and/or vitamin A agents (Muller, 1976; Scott, 1989), no clinical studies on the effects of fatty acid supplementation, feed change and showering on ichthyosis in Golden retrievers has been carried out to date.

Results show that a considerable proportion of dogs experience beneficial effects from the various symptomatic treatments. Overall nearly 50% of dogs have gained some degree of alleviation from one or more of the treatment options. It is difficult to estimate whether this is a true representation of the effects.

It could be argued that even more dogs could have gained positive effects, since some dogs have not been treated at all. In addition the type of feed used and the period of use, could also influence the effects. The same is the case for fish-oil supplements; there may be differences in the composition of fatty acids and quality of the supplements. The period of use is also important, Miller et al (2013c) recommends that fatty-acid supplementation be continued for 9-12 weeks before the effect should be assessed. The majority of owners have been supplementing the dogs for a period longer than this, thus no additional effects should be seen in these dogs.

Mauldin (2013) recommends using a moisturizer when showering in order to help regain barrier function. Results show that a much greater proportion of dogs that are showered in shampoo and moisturizer experience positive effects than in the dogs in general. Moreover, most owners didn't use keratolytic shampoo, as is also recommended by Mauldin (2013), this could cause even further underestimation of the true effect. The recommended frequency of showering could be as often as 2-4 times a week (Mauldin, 2013). None of the participating dogs were showered with this frequency.

In addition to the already discussed difficulties of estimating the true effects of treatments, some problems are the same among all three treatment-options. First, since the owners are not and cannot

be completely objective in their assessment of the effects, there may be some degree of discrepancy between what is the reality and what is responded in the questionnaire. Further the owners are not professionals, which could cause a positive effect to be overlooked or no effect to be assessed as a positive effect, simply because they don't have the clinical knowledge and know what to look for.

From the additional remarks made by some owners, it is evident that a number of the owners are very preconceived and have firm opinions on the effects of treatments. These owners may require greater improvements to acknowledge an effect than other owners or may completely refuse any effect irrespective of any actual effects.

Cadiergues and co-workers (2008) found no persistent improvement following the same and other treatments as in this current study. Whether the improvements seen in this study are persistent is not known, but since the treatments are only symptomatic, they most likely aren't. Improvement must to some extent rely on the owners' compliance with the treatment scheme, as Mauldin (2013) also concludes.

Overestimation of the proportions may be due to the problem of owners having to determine a possible effect and the fact that some of the dogs seem to show complete or partially remission without any supportive treatment. The supposed positive effect from changing the feeding might have occurred irrespective of the change of the feeding and so on. Even more it is not known whether these dogs had been subject to other treatments such as fatty acid supplementation or showering simultaneously with the change of feed. This could cause an overestimation of all of the treatment options in this study.

The responses to the first part of question 27 shows that the implications of ichthyosis to owners is dependent on the degree of scaling, as would be expected. The higher degree of scaling, the higher the degree of impact on the owner. The impact is, however, low in general.

The importance of a new Golden retriever having ichthyosis generally doesn't vary whether the owner currently owns a symptomatic or an asymptomatic dog. The majority of owners consider the ichthyosis status of a new dog of no or little importance. However if the degree of scaling of the current dog is taken into consideration, it is evident that the ichthyosis status of a new dog is more

important to the owners of dogs, which are severely scaling, than to the owners of dogs, which are not or mildly scaly.

It appears that among the owners of the participating Golden retrievers, there is a general wish to reduce the incidence of ichthyosis whether or not their dogs are symptomatic. The results show that the degree of scaling probably isn't the reason why owners wish for a reduction of the incidence of ichthyosis.

One could have expected that the majority of owners, who agree with a reduction, wouldn't want a new Golden retriever with ichthyosis. Conversely, 65% of owners, who agree with the reduction, also think it is of no or little importance whether a new dog has ichthyosis. This seem contradictory, since owners want the incidence reduced, but doesn't care whether a potential new dog has ichthyosis. The remaining 35% of owners indicate that the ichthyosis status of a new dog is highly significant, and are also predominantly the owners of the most severely affected dogs. This notion is more consequent, since these owners wish for a reduction and wouldn't want a new dog with ichthyosis.

The extra question 2 may uncover some of the reasoning behind the general wish for a reduction of the incidence of ichthyosis. A majority of owners mostly agreed with the answer option D. This statement specified that the incidence of all known inherited conditions, regardless of degree of implications, should be reduced. 2 owners, corresponding to 15%, believed that the implications on their dogs are too severe.

Moreover the owners, who mostly agreed with option D, are distributed among owners of asymptomatic dogs, mildly or severely scaling dogs. These notions could indicate that the wish for a reduction in the incidence, is not based on a concern for reduced welfare and life quality of these dogs, but on a more general perception of genetic disorders. This could imply that owners blindly would use DNA tests simply because they are available. This indiscriminate thought that because a genetic test is available, is should be used, emphasises one of the great problems in dog breeding – owners are most often not professionals and dogs breeding is often based the subjectivity of the breeder (Rooney and Sargan, 2010).

Reducing the incidence of ichthyosis through breeding would imply the use of genotyping of possible sires and dams. If DNA testing should be used as the foundation for selection of breeding animals, the test should be reliable to a degree, where being homozygous for a mutation causes development of disease. The findings in this study suggest that the mutation in the PNPLA-1 gene does not necessarily cause development of ichthyosis, and it may rather predispose to development of the condition. This apprehension is also mentioned in a Swiss study (Lipska-Owszarek, et al., 2011).

Furthermore, one could argue whether the mildly scaling dogs have ichthyosis at all, since ichthyosis is 'characterised by excessive scaling' (Cadiergues, et al., 2008). Thus, making use of the DNA test as the decision-maker even more questionable. If not used in the right way, DNA testing for ichthyosis could be a misguided attempt to ensure healthy animals, giving owners a false sense of security.

The Danish kennel Club have issued some ethical recommendations for breeding (Dansk Kennel Klub, 2014). One statement is that breeders should not use animals with heritable defects. Even if ichthyosis is regarded as a 'hereditary defect' and we should rely on the PNPLA-1 DNA test as the basis exclusion of possible breeding material, it would be very problematic for the Golden retriever breed in general. A very high allele frequency exists and a high number of Golden retrievers have been shown to be homozygous for the mutation. Excluding all of these dogs, significantly reduces the breeding population and hence may result in loss of genetic diversity, which has already been demonstrated (Calboli, et al., 2008).

As is seen in the results, there is a close kinship between most of the participating dogs. The degree of scaling can vary and is shown to vary within the same litter, but there is no overlapping between the asymptomatic and most severely scaling groups of dogs. This illustrates the problem with the DNA test for the PNPLA-1 mutation, some dogs display no symptoms, but littermates are scaling. The attempt to analyse the pedigree showed that the number of participating dogs simply is too low to demonstrate any clear tendencies, although several of the asymptomatic dogs were shown to be in close kinship, this could easily be caused by selection-bias.

Whether other genes are implicated in the development of ichthyosis or some specific environmental factors are involved, unfortunately cannot be concluded from this study. Mauldin and colleagues (2008) also hypothesised that other factors could be involved in the development of ichthyosis. In addition, the review of the pedigrees provided no clear evidence of some families or lines being more affected than others.

Whether additional genes are involved or the large variation of symptoms is caused by different environmental factors cannot be determined based on this study. Possibly, the mutation merely causes a predisposition to develop symptoms, rather than being a direct cause of the condition.

Basing an entire study on answers from individuals, who are personally invested, can significantly reduce the reliability of the study (Altman, 1991; Samuels and Witmer, 2003a).

First, the way the participating dogs were sampled, may cause some selection bias (Samuels and Witmer, 2003a). DNA testing for the PNPLA-1 mutation is voluntary and therefore may not represent the true population of Golden retriever that are homozygous for the mutation. Owners, who are having their dog genotyped, may for example consider ichthyosis as a serious disease, whereas owners of the opposite opinion may choose not to do the DNA testing, because they do not perceive ichthyosis as a problem.

Non-sampling bias, where the way a question is formulated can greatly influence answers (Samuels and Witmer, 2003a). This may be the reason for the ‘confusion’ in question 6. Here the word ‘abnormal’ may cause owners to respond no, despite the presence of scales, because owners may think of the scales as being ‘normal’ for their dog.

A serious problem with this survey is nonresponse bias (Samuels and Witmer, 2003a), where missing answers can influence the results greatly, since this is a small study, very few answers can shift the tendency. In addition to the missing answer in the returned questionnaires, a number of questionnaires were never returned from the owners. The problem is that the responders often are those with strong opinions or are very involved in the issue (Samuels and Witmer, 2003a).

8 Conclusion

As is concluded in other studies on ichthyosis, the variation of symptoms is very substantial. This current survey is the first study, where the subjects are chosen based on their genotype instead of the phenotype. This results in an even broader variation of symptoms. In this project a substantial number of completely asymptomatic dogs, which are homozygous for the alleged causative mutation, is presented. No studies have previously demonstrated completely healthy dogs, that are homozygous for the PNPLA-1 mutation.

Based on these findings, the DNA test for the PNPLA-1 mutation cannot be in implemented as a breeding restriction/recommendation of the Golden retriever breed, since this would exclude completely healthy dogs from the breeding population. For a Golden retriever to be homozygous for the PNPLA-1 mutation may merely result in a predisposition for development of ichthyosis rather than being the cause of ichthyosis.

Ichthyosis is not the most serious health issue in the Golden retriever breed and therefore shouldn't take first priority, when choosing breeding material. Excluding dogs with healthy hips, elbows and eyes from breeding, based on the PNPLA-1 DNA test, may not be beneficial for the general welfare and genetic diversity of the breed.

Although the proportion is small, some dogs show a high degree of symptoms and in some cases pruritus, which could be a threat to the welfare of the dogs. The incidence of such cases should be reduced. To date the knowledge of ichthyosis, the pathomechanisms and influence from genes and environmental factors are too negligible to base a breeding restriction upon. Instead owners should be encouraged to remove severely affected dogs from breeding in case some families or breeding lines are more affected than others.

The majority of the participating dogs are mildly impacted by the ichthyosis and a very small group of dogs are more severely scaling. Based on the owners replies, none of the dogs are severely impacted by the ichthyosis, despite the degree of scaling and presence of pruritus.

Positive effects from symptomatic treatment is evident in this survey and it should be attempted in dogs with symptoms of ichthyosis.

Ichthyosis' impact on the lives of owners is minimal, but is associated with the degree of symptoms in the dogs, as would be expected. If purchasing a new Golden retriever the ichthyosis status is generally unimportant to the owners. Despite this, a majority of the owners agree that the incidence of ichthyosis should be reduced through breeding. The reason is not completely clear, but it was indicated that owners want to reduce the incidence of any inherited condition regardless of the impact on the animals' welfare.

To summarize, there is a considerable variation in the clinical manifestations of ichthyosis. A substantial number of Golden retrievers that are homozygous for the PNPLA-1 mutation are completely free of symptoms of ichthyosis. On this background a breeding restriction based on genotyping for this mutation cannot be recommended.

9 Perspectives

Based on the conclusion, it could be stated that it is impossible to reduce the incidence of ichthyosis, since no breeding restriction based on the PNPLA-1 mutation can be recommended, and since exclusion of homozygous 'affected' dogs would cause a high number of dogs to be excluded. It is however problematic that when there is no restriction, breeders may lack an incentive to discontinue the use of highly symptomatic dogs.

At the present owners should be advised to end using dogs that are showing severe signs of ichthyosis. Exclusion of the worst affected individuals is also practised in cases of hip dysplasia, elbow dysplasia heart conditions and so on.

In order to implement a restriction on ichthyosis on the long term basis, more knowledge on the factors that influence the development of symptoms must be acquired. To date the pathomechanism behind the scaling is merely perceived and no studies have mentioned the mechanism behind the hyperpigmentation.

New studies on the degree of scaling and hyperpigmentation in a cross-section of the real Golden retriever population should be done. The question is whether dogs that are not homozygous for the mutation, could display symptoms of ichthyosis. In addition a more in-depth study of the pedigrees of the Golden retrievers should be done in order to demonstrate whether additional genes are involved.

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Appendix 1 – The questionnaire

Dato: _____

Spørgeskema om ichthyosis hos Golden retriever

1) Generelle oplysninger om din hund_____

Er hunden kastreret / steriliseret?	JA ☐	NEJ ☐
Bor hunden primært ude eller inde?	UDE ☐	INDE ☐
Pelstype	Glat ☐	Bølget ☐
Har hunden fået konstateret symptomer på ichthyosis hos en dyrlæge?	JA ☐	NEJ ☐
- hvis JA, angiv symptomer samt dyrlæge	Symptomer: ☐ Skæl ☐ Mørkfarvning af huden ☐ Tør hud ☐ Andet, beskriv: _____ _____ Dyrlæge/klinik: _____	

Kliniske symptomer

Hvis din hund har kliniske symptomer på ichthyosis besvares punkt 2-14, ellers fortsættes til punkt 15.

2) Hvornår oplevede du første gang at hunden havde symptomer på ichthyosis?

☐ Skæl (angiv alder: _____ år _____ måneder)

☐ Mørkfarvning af huden (angiv alder: _____ år _____ måneder)

☐ Tør hud (angiv alder: _____ år _____ måneder)

☐ Andet, beskriv: _____ (angiv alder: _____ år ____ måneder)

3) Har din hunds symptomer været på samme niveau siden da, eller er der sket en udvikling i symptomerne:

☐ Symptomerne har været nogenlunde på samme niveau, siden de startede

☐ Symptomerne er blevet mere udtalte/værre siden de startede

☐ Symptomerne er blevet mindre udtalte/bedre siden de startede

4) Hvilken årstid oplever du at din hunds symptomer er værst?

☐ Vinter

☐ Forår

☐ Sommer

☐ Efterår

☐ Symptomerne er lige udtalte hele året, der sker ingen ændring i forhold til årstider.

5) Har din hund siden den fik symptomerne haft en eller flere helt symptomfri perioder? ☐ JA ☐ NEJ

Hvis JA, beskriv hvornår (hundens alder, og periodens varighed): _____

_____

Hvis JA, blev der gjort noget anderledes i forhold til hunden i denne/disse periode(r), f.eks. en ny slags børste, ophold et andet sted, som kunne forklare symptomfriheden? Beskriv:

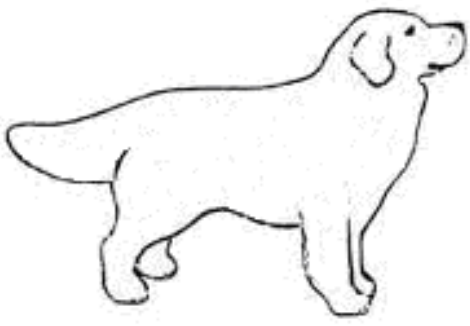
_____

Skæl

6) Har din hund på nuværende tidspunkt eller tidligere i sit liv haft tendens til unormal skæl-dannelse (hos en normal hund kan der ses enkelte små løse skæl i perioder)? ☐ JA ☐ NEJ

Hvis JA besvares punkterne 7-11
Hvis NEJ springes direkte til punkt 12

7) Udbredelse af skæl (skraver områder hvor din hund har/har haft skæl



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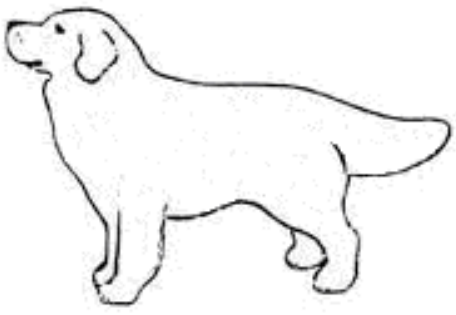

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8) Angiv farven af skællene:

☐ Hvid
☐ Lys grå
☐ Mørk grå
☐ Sort
☐ Anden farve (angiv): _____

9) I hvilke situationer oplever du at din hund taber skæl fra huden/pelsen?

☐ Skæl drysser fra hundens hud/pels spontant
☐ Skæl drysser fra hundens hud/pels hvis jeg klør/aer hunden meget
☐ Skæl sidder i børsten efter børstning/kæmning
☐ Skæl falder af efter badning
☐ Andre situationer (angiv hvilke): _____

Eventuelle kommentarer: _____

_____

10) Hvor observerer du skæl:

☐ Skæl kan ses på hunden spontant, uden at sprede/dele pelsen
☐ Skæl kan ses hvis pelsen spredes/deles og huden/pelsen nærstuderes
☐ Der ses skæl i hundens kurv/sovested
☐ Der ses skæl på gulve hvor hunden har været
☐ Der ses skæl andre steder (angiv hvor): _____

Eventuelle kommentarer: _____

_____

11) Hvordan vil du selv graduere din hunds skællen (markér på den grå bjælke med et X):

Ingen skæl Moderat mængde skæl Stor mængde skæl

0 1 2 3 4 5 6 7 8 9 10

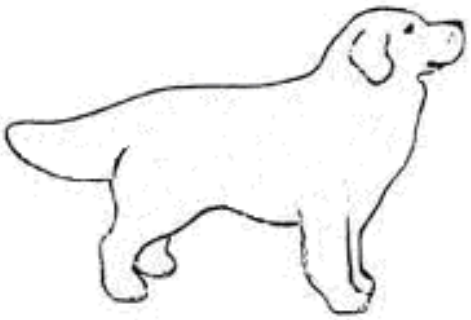
Mørkfarvning af huden

12) Har din hund på nuværende tidspunkt eller tidligere i sit liv haft mørkfarvning/sortfarvning af områder i huden? ☐ JA ☐ NEJ

Hvis JA, er hunden blevet undersøgt af en dyrlæge pga. mørkfarvningen i huden? Angiv dyrlæge og diagnose: _____

Hvis JA i punkt 12 besvares punkterne 13-14
Hvis NEJ i punkt 12 springes direkte til punkt 15

13) Udbredelse af mørkfarvning i huden (skraver områder hvor din hund har/har haft mørkfarvning af huden)



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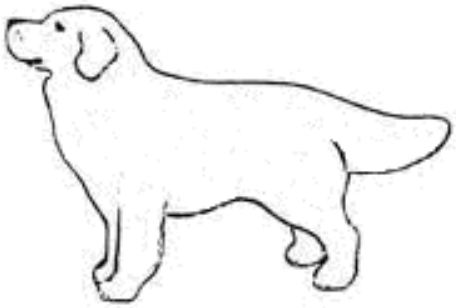

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14) Hvordan vil du selv graduere intensiteten af din hunds mørkfarvning i huden (markér på den grå bjælke med et X):

Ingen mørkfarvning — Moderat mørkfarvning — Kraftig mørkfarvning

0 1 2 3 4 5 6 7 8 9 10

15) Hvor meget vurderer du, at din hund er påvirket af sin ichthyosis? (markér på den grå bjælke med et X)

Ingen påvirkning — Moderat påvirkning — Meget stor påvirkning

0 1 2 3 4 5 6 7 8 9 10

Behandling

16) Modtager din hund nogen behandling pga. skæl eller mørkfarvning i huden? ☐ JA ☐ NEJ

Hvis JA, angiv hvilken behandling: _____

Foder

17) Hvilket foder anvender du til din hund (mærke og foderets eksakte navn):

_____

18) Hvis din hund har/har haft tendens til skæl eller mørkfarvning i huden, har du da oplevet at skift af foder har haft effekt på dette? ☐ slet ikke ☐ nogen effekt ☐ god effekt

Hvis JA, beskriv effekten: _____

Hvilke(t) foder har haft god effekt: _____

Hvilke(t) foder har haft dårlig effekt: _____

Fiskeolier

19) Har din hund på noget tidspunkt i sit liv fået fiskeolier? ☐ JA ☐ NEJ

-Hvis JA, hvilken alder havde hunden da han/hun fik fiskeolie? _____ år _____ måneder

-I hvor lang tid fik hunden fiskeolie? _____ måneder

Hvis din hund har/har haft tendens til skæl eller mørkfarvning i huden, har du da oplevet at fiskeolier har haft effekt på dette? ☐ slet ikke ☐ nogen effekt ☐ god effekt

Beskriv effekten: _____

Hvilken fiskeolie har været anvendt? (eksakte navn) _____

Shampoo/balsam

20) Vasker du din hund i shampoo og balsam? ☐ Shampoo ☐ Balsam ☐ Ingen af delene

Hvis JA, angiv det eksakte navn på shampoo og/eller balsam: _____

21) Hvis din hund har/har haft tendens til skæl eller mørkfarvning i huden, har du da oplevet en effekt af shampoo/balsam på dette? ☐ slet ikke ☐ nogen effekt ☐ god effekt

Hvis JA, beskriv effekten: _____

Hvor ofte vaskes din hund: _____

Øvrige

22) Har din hund i løbet af sit liv haft problemer med hårtab? ☐ JA ☐ NEJ

23) Har din hund i løbet af sit liv haft problemer med kløe (dvs. hvor hunden afbryder den aktivitet den er igang med, spise/lege/sove, for at begynde at klø, slikke eller gnubbe sig? -eller hvor hunden har kløet, slikket eller gnubbet sig så meget at der kom sår i huden)? ☐ JA ☐ NEJ

-Er årsagen til kløen blevet identificeret? ☐ JA ☐ NEJ Angiv årsag: _____

24) Har din hund i løbet af sit liv haft hudbetændelse, eller andre hudsygdomme, som er blevet konstateret hos en dyrlæge? ☐ JA ☐ NEJ

-hvis JA, angiv diagnose og dyrlæge:

_____

_____

_____

25) Har din hund i løbet af sit liv haft andre heldbredsmæssige problemer? ☐ JA ☐ NEJ

-hvis JA, angiv hvilke: _____

Eventuelle kommentarer: _____

_____

_____

26) Modtager din hund nogle andre former for behandling (angiv hvilke, f.eks. Rimadyl® Vet. mod gigt etc.)?

_____

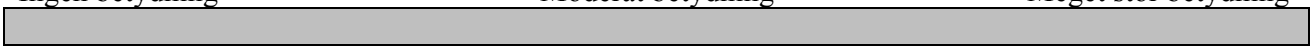
Har din hund modtaget andre behandlingsformer som har haft effekt mod ichthyosis? _____

_____

Ichthyosis' betydning for dig og din hund

27) Hvor stor betydning har det for dig at din hund har ichthyosis? (Markér på bjælken med et X)

Ingen betydning — Moderat betydning — Meget stor betydning



0 1 2 3 4 5 6 7 8 9 10

Nedenfor følger nogle udsagn, som gruppen bag spørgeskemaet er stødt på i arbejdet med ichthyosis hos Golden retriever, fra ejere og andre personer. Markér hvor enig/uenig du er i hvert af de følgende udsagn:

	Helt uenig	Delvis uenig	Hverken/eller	Delvis enig	Helt enig
- Jeg tænker ofte over at min hund har ichthyosis	☐	☐	☐	☐	☐
- Jeg gør ekstra rent pga. de skæl min hund taber	☐	☐	☐	☐	☐
- Jeg undlader at tage min hund med på besøg i andres hjem, fordi den skæller	☐	☐	☐	☐	☐
- Jeg lukker min hund ind i et andet rum når vi har gæster fordi den skæller meget	☐	☐	☐	☐	☐
- Hvis jeg havde vidst at min hund havde ichthyosis havde jeg ikke købt den	☐	☐	☐	☐	☐
- Jeg er bekymret for om skællene giver øget risiko for udvikling af allergi eller anden sygdom hos børn eller andre familiemedlemmer	☐	☐	☐	☐	☐
- Jeg børster min hund mere end jeg ellers ville gøre pga. dens skæl	☐	☐	☐	☐	☐
- Jeg vasker min hund oftere end jeg ellers ville gøre pga. dens skæl	☐	☐	☐	☐	☐
- Ichthyosis er arvelig, og jeg synes man skal forsøge at reducere forekomsten via avlen	☐	☐	☐	☐	☐
- Jeg oplever at omgivelserne ser anderledes på min hund og mig pga. ichthyosis	☐	☐	☐	☐	☐

28) Hvis/når du skal have en ny hund, vil du da vælge en Golden retriever igen?

☐ Ja, helt sikkert ☐ Ja, højst sandsynligt ☐ Nej, sandsynligvis ikke ☐ Nej, helt sikkert ikke ☐ Ved ikke

Hvis NEJ, angiv årsagen: _____

29) Hvis du skal have en Golden retriever igen, har det da betydning for dig om hunden har ichthyosis?

☐ Ingen betydning

☐ Lidt betydning

☐ Stor betydning

☐ Meget stor betydning

30) Hvor vidt vil du karakterisere ichthyosis som et kosmetisk problem versus en sygdom (Kryds af, og markér efterfølgende med et X på den grå bjælke)

- Jeg vil karakterisere ichthyosis som et kosmetisk problem? ☐ JA ☐ NEJ

Hvis JA, hvordan vil du graduere problemet? (Markér på den grå bjælke med et X)

Lille kosmetisk problem Moderat kosmetisk problem Stort kosmetisk problem

0 1 2 3 4 5 6 7 8 9 10

- Jeg vil karakterisere ichthyosis som en sygdom? ☐ JA ☐ NEJ

Hvis JA, hvordan vil du graduere sygdommen? (Markér på den grå bjælke med et X)

Uden betydning Moderat betydning Meget stor betydning

0 1 2 3 4 5 6 7 8 9 10

Mange tak for din deltagelse i undersøgelsen.

I forbindelse med bearbejdelsen af spørgeskemaerne kan vi eventuelt få brug for at kontakte dig med uddybende spørgsmål.

□ Ja, I må gerne kontakte mig på tlf/mail: _____

□ Nej, jeg ønsker ikke at blive kontaktet i forbindelse med denne undersøgelse

Appendix 2 - Answers

Hund	Køn	1: Neutraliseret	1: Lever ude eller inde	1: Pelstype	1: Symptomer konstateret hos dyrlæge	1: Symptomer	2: Skæl-alder
A	Hanhund	Nej	Inde	Glat	Nej	-	-
B	Tæve	Ja	Inde	Bølget	Nej	-	4-6 mdr
C	Tæve	Nej	Inde	-	Ja	Skæl	1 mdr
D	Hanhund	Nej	Inde	Glat	Ja	Skæl, mørkfarvning, tør hud	6 mdr
E	Tæve	Nej	Inde	-	Ja	Skæl, mørkfarvning	1-2 mdr
F	Tæve	Nej	Inde	Bølget	Nej	-	-
G	Hanhund	Nej	Inde	-	-	-	2 mdr
H	Hanhund	Nej	Inde	Bølget	Ja	Skæl, mørkfarvning, tør hud	2 mdr
I	Hanhund	Nej	Inde	Bølget	Ja	Skæl, mørkfarvning	96 mdr
J	Tæve	Nej	inde	Glat	Ja	Skæl	2 mdr
K	Tæve	Nej	Inde	Bølget	Ja	Skæl, mørkfarvning	-
L	Tæve	Nej	Inde	Bølget	Ja	Skæl, mørkfarvning	2 mdr
M	Hanhund	Nej	Inde	Bølget	Nej	-	-
N	Hanhund	Nej	Inde	Bølget	Nej	-	-
O	Hanhund	Nej	Inde	Bølget	Ja	Skæl, mørkfarvning	2 mdr
P	Tæve	Nej	Inde	Bølget	Nej	-	-
Q	Hanhund	Nej	Inde	Glat	Ja	Skæl, mørkfarvning	2 mdr
R	Tæve	Nej	Inde	Bølget	Ja	Skæl, mørkfarvning + kløe og sår	10 mdr
S	Tæve	Ja	Inde	Bølget	Nej	-	-
T	Tæve	Nej	Inde	Glat	Nej	-	-
U	Tæve	Nej	Inde	Glat	Nej	-	2 mdr
V	Tæve	Nej	Inde	Bølget	Nej	-	-
W	Tæve	Nej	Inde	Glat	Ja	Skæl, mørkfarvning	13 mdr
X	Hanhund	Nej	Inde	Bølget	Nej	-	2 mdr
Y	Hanhund	Nej	Inde	Glat	Nej	-	-
Z	Tæve	Nej	Inde	Glat	Ja	Skæl, mørkfarvning, tør hud, kløe	4 mdr
Æ	Hanhund	Nej	Inde	Glat	Nej	-	-
Ø	Tæve	Ja	Inde	Glat	Ja	Skæl, mørkfarvning	3 mdr
Å	Tæve	Nej	Inde	Bølget	Nej	-	-
AA	Tæve	Nej	Inde	Bølget	Nej	Skæl	1 mdr
BB	Tæve	Nej	Inde	Glat	Ja	Skæl	6 mdr
CC	Hanhund	Nej	Inde	Glat	Ja	Skæl, mørkfarvning	1 mdr
DD	Hanhund	Nej	Inde	Bølget	Ja	Skæl, mørkfarvning	1 mdr
EE	Hanhund	Nej	Inde	Bølget	Ja	Skæl, mørkfarvning	-
FF	Hanhund	Nej	Inde	Glat	Ja	Skæl, mørkfarvning, tør hud	2,5 mdr
GG	Tæve	Nej	Inde	Glat	Ja	Skæl + kløe	30 mdr

Hund	2: Mørkfarvning - alder	2: Tør hud - alder	2: Andet - alder	3:Udvikling i symptomer	4: Værste årstid	5: Symptomfri perioder	5: Hvad blev gjort anerledes
A	-	-	-	-	-	-	-
B	-	4-6 mdr	-	Samme niveau	Forår/efterår	Nej	-
C	-	-	-	Samme niveau	Lige udtalte	Ja, 10 mdr gammel i 3 mdr	Ingenting
D	6 mdr	6 mdr	-	Samme niveau	Lige udtalte	Nej	-
E	2 mdr	-	-	Mindre/ bedre	-	Ja, skæl forsvandt da hunden blev 6 mdr	Intet
F	-	-	-	-	-	-	-
G	-	2 mdr	-	Mindre/ bedre	Lige udtalte	Nej	-
H	2 mdr	2 mdr	-	Mindre/ bedre	Lige udtalte	Ja, som 6 mdr gammel forsvandt farve og tørhed i huden	Bliver beh. Med dermashine og vasket med D-Calm
I	96 mdr	-	-	Samme niveau	Lige udtalte	Nej	-
J	-	-	-	Samme niveau	Lige udtalte	Ja	Beh. Med Shampoo, foder, Barf og olie
K	-	-	-	Mindre/ bedre	Forår	Ja, symptomfri periode på 14 mdr	-
L	2 mdr	-	-	Samme niveau	Lige udtalte	Nej	-
M	-	-	-	-	-	-	-
N	-	-	-	-	-	-	-
O	4 mdr	-	-	Mere/værre	Lige udtalte	Nej	-
P	-	-	-	-	-	-	-
Q	6 mdr	-	-	Samme niveau	Lige udtalte	Nej	-
R	10 mdr	-	12 mdr	Mindre/ bedre	Lige udtalte	Nej	-
S	-	-	-	-		-	-
T	-	-	-	-	-	-	-
U	-	-	-	Mindre/ bedre	Lige udtalte	Nej	-
V	12 mdr	-	-	-	-	-	-
W	13 mdr	-	-	Samme niveau	Lige udtalte	Nej	-
X	-	-	-	Mindre/ bedre	Lige udtalte	Nej	-
Y	-	-	-	-	-	-	-
Z	4 mdr	4 mdr	24 mdr	Samme niveau	Lige udtalte	Nej	-
Æ	-	-	-	-	-	-	-
Ø	12 mdr	-	-	Mindre/ bedre	Lige udtalte	Ja, ved 2 års alderen og frem	Foderskift
Å	-	-	-	-	-	-	-
AA	-	-	-	Mindre/ bedre	-	Ja	Har skæl i forbindelse med fældning
BB	-	-	-	Samme niveau	-	Ja	Kun skæl i forbindelse med fældning
CC	12 mdr	-	-	Mindre/ bedre	Vinter, forår, efterår	Nej	-
DD	12 mdr	1 mdr	-	Mindre/ bedre	Lige udtalte	Nej	-
EE	-		-	Samme+ mindre/bedre	Lige udtalte	Nej	-
FF	3 mdr	3 mdr	-	Mere/værre	Lige udtalte	Nej	-
GG	30 mdr	-	-	-	Lige udtalte	Nej	-

Hund	6: Unormal skældannelse	8: Farve af skæl	9: Tab af skæl	10: Hvor observeres skæl	11: Graduering af skællen
A	-	-	-	-	-
B	Ja	Lys grå	Børstning	Pelsen deles, hvor hunden har været	Moderat -6,5
C	Nej	Hvid	Klør/aer	Hvor hunden har været	-
D	Ja	Lys grå, mørk grå	Klør/ aer, børstning	Spontant, kurv/ sovested, hvor hunden har været	Svær/meget svær - 8
E	Nej	Hvid, lys grå, mørk grå	Klør/ aer, børstning	Pelsen deles	Ingen/ mild 0
F	-	-	-	-	-
G	Ja	Hvid	Klør/aer	Pelsen deles	Moderat - 4
H	Ja	Hvid	Børstning	Pelsen deles	Moderat - 4
I	Ja	Hvid, Lys grå	Klør/ aer, børstning	Pelsen deles	Ingen/mild - 3
J	Ja	Lys grå, mørk grå	Spontant, klør/aer, Børstning	Spontant, pelsen deles, kurv/ sovested, hvor hunden har været	Svær/meget svær - 8
K	Ja	Sort	Klør/aer	Pelsen deles	Ingen/mild - 2
L	Ja	Hvid, sort	Klør/ aer, børstning	Pelsen deles, hvor hunden har været	Moderat - 5
M	-	-	-	-	-
N	-	-	-	-	-
O	Ja	Mørk grå	Spontant, klør/aer, børstning, efter badning	Spontant, pelsen deles, kurv/ sovested, hvor hunden har været	Svær/meget svær - 9
P	-	-	-	-	-
Q	Nej	Hvid	Børstning	Pelsen deles	Ingen/mild - 2
R	Ja	Hvid, Lys grå	Spontant, kløer/aer, børstning, efter badning	Spontant, pelsen deles, kurv/ sovested, hvor hunden har været	Moderat - 4,5
S	-	-	-	-	-
T	-	-	-	-	-
U	Nej	Hvid	Spontant	Pelsen deles, kurv/ sovested	Moderat - 5
V	-	-	-	-	-
W	Ja	Mørk grå	Spontant, børstning	Spontant, kurv/ sovested, hvor hunden har været	Moderat 6,75
X	Ja	Hvid	Børstning	Pelsen deles	Ingen/ mild - 1
Y	-	-	-	-	-
Z	Ja	Lys grå, mørk grå	Spontant, kløer/aer, børstning, efter badning	Spontant, hvor hunden har været	Svær/ meget svær - 8
Æ	-	-	-	-	-
Ø	Ja	Hvid, lys grå	Børstning, efter badning	Pelsen deles	Moderat - 5
Å	-	-	-	-	-
AA	Ja	Lys grå	Børstning	Pelsen deles	Ingen/ mild - 1
BB	Nej	-	-	-	-
CC	Ja	Mørk grå	Børstning	Pelsen deles	Moderat - 6
DD	-	Mørk grå	Børstning	Pelsen deles	Moderat - 5
EE	Ja	Lys grå	Børstning	Pelsen deles	Ingen/ mild - 3
FF	-	Mørk grå, sort	Spontant, klør/aer, Børstning	Spontant, pelsen deles, kurv/ sovested, hvor hunden har været	Svær/meget svær - 10
GG	Ja	Lys grå	Spontant, klør/aer, børstning, efter badning	Pelsen deles, kurv/ sovested, hvor hunden har været+ tøj	Moderat - 6

Hund	12: Mørkfarvning	14: Graduer mørkfarvning	15: Hundens påvirkning	16: Behandling	17: Foder	18: Effekt af foderskift
A	Ja	-	Ingen/mild - 0	Nej	-	God effekt
B	Ja	-	Moderat - 5	Nej	-	Nogen effekt
C	Nej	-	Ingen/mild - 0,5	Nej	Essential	Slet ikke
D	Ja	Moderat - 5	Ingen/mild - 4	Ja, olie (også i pels), urter	Hills J/d	Slet ikke
E	Ja	-	Ingen/mild - 0	Nej	Pedigree ative m/ kylling	-
F	-	-	Ingen/mild - 2	Nej	Diafarm, Diadog, maintanance large breed	Slet ikke
G	Nej	-	Ingen/mild - 2	Nej	Olivers selected Fish/ Grøn kalun	Slet ikke
H	Ja	Svær/meget svær - 7,5	Ingen/mild - 1	Nej	Royal canin og hills til large breed	Slet ikke
I	Ja	Ingen/mild - 3	Ingen/mild - 1	Nej	Barf	God effekt
J	Ja	Ingen/mild - 1	Ingen/mild - 0	Ja, foder + shampoo	Golden eagle holistic health	God effekt
K	Ja	Svær/meget svær - 9	Ingen/mild - 1	Nej	Pedigree vital adult	-
L	Ja	Moderat - 3,5	Ingen/mild - 0	Nej	Flatazor Elite sensible	Slet ikke
M	-	-	Ingen/mild - 1	Nej	Royal canin Maxi Junior	-
N	-	-	Ingen/mild - 0	Nej	Hills + Chappi eller Chrisco Knas	-
O	Ja	Svær/meget svær - 7	Ingen/mild - 0	Nej	Purina-Proplan (forskellige varianter)	Slet ikke
P	-	-	Ingen/mild - 0	Nej	Taste of the wild pacific stream canine forumla	Slet ikke
Q	-	-	-	Nej	Eukanuba custom care - sensitive skin & coat	Nogen effekt
R	Ja	Moderate - 6	Ingen/mild - 0	Nej	BARF	God effekt
S	-	-	Ingen/mild - 0	Nej	Hills j/d	-
T	-	-	Ingen/mild - 0	Nej	RC. Adult LB	-
U	Nej	-	Ingen/mild - 0	Nej	Eukanuba Golden retriever	Slet ikke
V	Ja	Moderat - 5	Ingen/mild - 1	Nej	SP /j/d	Slet ikke
W	Ja	vær/meget svær - 8,5	Ingen/mild - 3	Nej	Eukanuba, adult large breed, lamb/rice	Slet ikke
X	Nej	-	Ingen/mild - 0	Nej	Fromm adult gold	-
Y	Ja	Ingen/mild - 3	Ingen/mild - 0	Nej	RC maxi junior + Royal canin maxi adult + Rc energy 4300	Slet ikke
Z	Ja	Svær/meget svær - 7	Moderat - 6	Nej	Taste of the wild - fisk	God effekt
Æ	-	-	Ingen/mild - 0	Nej	Eukanuba	-
Ø	Ja	Moderat - 5	Ingen/mild - 3	Nej	Olivers selected fish medi	God effekt
Å	-	-	Ingen/mild - 0	Nej	Eukanuba Golden retriever	-
AA	Nej	-	Ingen/mild - 0	Nej	Virbac adult / Royal canin adult	Slet ikke
BB	Nej	-	Ingen/mild - 0	Nej	Royal canine Satiety	Slet ikke
CC	Ja	Moderat - 4	Ingen/mild - 0	Nej	Eukanuba Adult LB	Slet ikke
DD	Ja	Moderat - 5	Ingen/mild - 0	Nej	Royal Canin adult large breed	Nogen effekt
EE	Ja	Ingen/mild - 3	Ingen/mild - 0	Nej	Dermatosis - EUD	Slet ikke
FF	Ja	Svær/meget svær - 10	Moderat - 5	Nej	RC hypoallergenic	Slet ikke
GG	Nej	-	Ingen/ mild - 0-3	Nej	Wolfsblut	-

Hund	18: Effekt, hvis ja	18: Foder med god effekt	18: Foder med dårlig effekt	19: Fiskeolie	19: Alder ved fiskeolie	19: Periode med fiskeolie
A	Skælen næsten væk	Barf	Korn produkter i fodret	Ja	12 mdr	3-4 mdr
B	-	-	-	Ja	6 mdr	6-12 mdr
C	-	-	-	-	-	-
D	-	-	-	Ja	30 mdr	6 mdr
E	Har ikke skiftet foder	-	-	Nej	-	-
F	Vi søger for at give olie & godt foder og har derfor ikke haft problemer med skæl?	-	-	Ja	2 mdr	Altid
G	-	-	-	Ja	12 mdr	Den får stadig
H	-	-	-	Nej	-	-
I	Næsten ingen skæl	Barf	Hills/ royal canin	Ja	96 mdr	6 mdr
J	Skæl forsvinder næsten helt	Eukanuba dermatosis, BARF, Golden eagle	Royal canin golden retriever	Ja	6 mdr	-
K	-	-	-	nej	-	-
L	-	-	-	Ja	12 mdr	2 mdr
M	-	-	-	Nej	-	-
N	-	-	-	Nej	-	-
O	-	-	-	Ja	4 mdr	12 mdr
P	-	-	-	Nej	-	-
Q	Mindre mørk og mindre ' udslæt' ved lysken	Eukanuba custom care - sensitive skin	Foder der ikke tager hensyn til hud og pels	Nej	-	-
R	Sår fosvinder, pels bliver pæn, mørkfarvning og skæl minimeres	Barf	Alt tørfoder	Ja	9 mdr	28 mdr
S	-	-	-	Nej	-	-
T	-	-	-	Nej	-	-
U	-	-	-	Nej	-	-
V	-	-	-	Ja	6 mdr	3 mdr
W	-	-	-	Nej	-	-
X	-	-	-	Nej	-	-
Y	-	-	-	Ja	28 mdr	6
Z	Blankere pels, mindre skæl, mindre kløe	Taste of the wild - fisk	Alt andet	Ja	12 mdr	12 mdr
Æ	-	-	-	Nej	-	-
Ø	Stor reducering af skæl	Olivers selected fish medi	Hills til golden retriever	Ja	3 mdr	24 mdr
Å	-	-	-	Nej	-	-
AA	-	-	-	Ja	2 mdr	-
BB	-	-	-	Ja	4 mdr	Altid
CC	-	-	-	Ja	1 mdr	altid
DD	-	-	Eukanuba	Ja	Altid	Altid
EE	-	-	-	Ja	11 mdr	Altid
FF	-	-	-	Ja	14 mdr	3 mdr
GG	-	-	-	Ja	24 mdr	10 mdr

Hund	19: Effekt af fiskeolie	19: Effekt	19: Navn på fiske olie	20: Vask	20: Shampoo/Balsam	21: Effekt af shampoo/ balsam
A	Slet ikke	-	-	Shampoo	Isle of dogs	Slet ikke
B	Slet ikke	-	-	Shampoo	-	God effekt
C	-	-	-	-	-	-
D	Slet ikke	-	Kronch lakseolie	Shampoo	Brun sæbe	Slet ikke
E	-	-	-	Shampoo	Kw mink olie	Slet ikke
F	God effekt	Næsten ingen skæl	Olivers omega 3 boost	Shampoo	Karlie puppy shampoo	Nogen effekt
G	Slet ikke	-	-	Shampoo + balsam	Bundgaard & Bluhme	Nogen effekt
H	-	-	-	Shampoo + balsam	Dermashine balsam, D-calm shampoo	God effekt
I	Nogen effekt	-	Dog Fish Salmon (omega 3)	Shampoo	Aloe Vera fra KW	Nogen effekt
J	God effekt	Skæl forsvinder næsten helt	Eforion olie, Henne pet Kronch lakseolie	Shampoo	Keratolux	God effekt
K	-	-	-	Shampoo	-	Slet ikke
L	Slet ikke	-	Vildtlakseolie fra alaska	Shampoo + balsam	Isle of dogs	Slet ikke
M	-	-	-	Ingen af delene	-	-
N	-	-	-	Ingen af delene	-	-
O	Slet ikke	.	-	Shampoo	Tropiclean Aloe moist	Slet ikke
P	-	-	-	Shampoo	-	-
Q	-	-	-	Ingen af delene	-	-
R	Slet ikke		Forskellige	Shampoo	Keratolux + malaseb	Slet ikke
S	-	-	-	Shampoo	Dogman shampoo	-
T	-	-	-	Shampoo	Dogman shampoo	-
U	-	-	-	Ingen af delene	-	-
V	Nogen effekt	Skællede som hvalp, synes det hjalp lidt	Megaderm fra virbac	Shampoo	Virbac alm shampoo	Slet ikke
W	-	-	-	Ingen af delene	-	-
X	-	-	-	Shampoo	KW citron shampoo	Slet ikke
Y	Slet ikke	-	Hike Tobis Olie	Shampoo+ Balsam	Loreal	Slet ikke
Z	God effekt	Mindre skæl	Olivers omega 3 boost	Shampoo + balsam	Allerderm moist + humilac spray	Nogen effekt
Æ	-	-	-	Shampoo	KW minkolie shampoo	-
Ø	Slet ikke	-	-	Shampoo + balsam	Furminator deshedding + humilac	God effekt
Å	-	-	-	Ingen af delene	-	-
AA	Slet ikke	-	-	Shampoo	KW Minkolie	Slet ikke
BB	Slet ikke	Vides ikke, har fået det altid	Dr. Baddaky/ viacutan/ lakseolie	Shampoo	KW citron	Slet ikke
CC	-	Vides ikke, har altid fået	Dr. Baddaky/ viacutan/ lakseolie	Shampoo	KW Minkolie	Slet ikke
DD	-	Vides ikke, har altid fået	Dr. Baddaky/ viacutan/ lakseolie	Shampoo	KW minkolie/ Virbac epi soothe	Slet ikke
EE	-	Vides ikke, har altid fået	Lakseolie	Shampoo	KW Mink olie	Slet ikke
FF	Slet ikke	-	Viacutan	Shampoo + balsam	Allerderm moist virbac	Nogen effekt
GG	Slet ikke	-	Olivers	Shampoo	Wheat shampoo/ allerderm pro	Slet ikke

Hund	21: Hvis ja, effekt	21: Hvor ofte vaskes	22: Hårtab	23: Kløe	23: Årsag identificeret	24: Andre hudsygdomme	24: Diagnose
A	-	Kun til udstilling	Nej	Nej	-	Nej	-
B	-	hver 4-6 mdr	Ja	Ja	Nej	Husstøvmideallergi	Nej
C	-	-	Nej	Nej	-	Nej	-
D	-	3 gange pr år	Nej	Nej	-	Nej	-
E	-	3-4 gange om året	Nej	Nej	-	Nej	-
F	Øget skæl efter vask som hvalp	hver 4-6 mdr	Nej	Ja	Nej	Nej	-
G	Fjerner løs skæl	Efter behov	Nej	Nej	-	Ja	Hårsækmider + kronisk ørebetændelse
H	Huden blev blød og 'normal', ikke tør og skællet.	Ved behov.	Nej	Nej	-	Nej	-
I	Skællene bliver mindre	2-3 gange årligt	Nej	Nej	-	Nej	-
J	Skæl forsvinder næsten helt	3-4 gange om måneden	Nej	Nej	-	Nej	-
K	-	4 gange årligt	Nej	Ja	Ja, en rift	Ja	kradsede sig, fik betændelse, fik penicillin og det gik væk
L	-	Hver 3 mdr	Nej	Nej	-	Ja	-
M	-	Aldrig	Nej	Nej	-	Nej	-
N	-	Aldrig	Nej	-	-	Ja	Hotspot
O	-	Hver halve år	Nej	Nej	-	Nej	-
P	-	2-3 x årligt	Nej	Nej	-	Nej	-
Q	-	-	Nej	Ja	Nej	Ja	Udslæt i lysken
R		Efter behov - et par gange årligt	Ja	Ja	Ja, ichthyosis	Nej	
S	-	ca. hver halve år eller ved behov	Nej	Nej	-	Nej	-
T	-	ca. hver halve år eller ved behov	Nej	-	-	Nej	-
U	-	-	Nej	Nej	-	Nej	-
V		1 x pr mdr	Nej	Nej	-	Nej	-
W	-	-	Nej	Nej	-	Nej	-
X	-	1 gang årligt	Nej	Nej	-	Nej	-
Y	-	1-2 gange årligt	Nej	Nej	-	Nej	-
Z	Færre skæl i dagene efter vask	1x pr 3 uger	Nej	Ja	Nej	Nej	-
Æ		8-12 gange årligt	Nej	Nej	-	Ja	Hotspot
Ø	-	Efter behov	Nej	Ja	ja, stressede situationer	Nej	-
Å	-	-	Nej	Nej	-	Nej	-
AA	-	Ca. hver 2-3 mdr	Nej	Nej	-	Nej	-
BB	-	1 gang hver 3-4 mdr	Nej	Nej	-	Nej	-
CC	-	1xmdr	Nej	Nej	-	Nej	-
DD	-	ca hver 3. mdr	Nej	Nej	-	Nej	-
EE	-	1 gang pr mdr	Nej	Nej	-	Nej	-
FF	-	2 gange om mdr	Nej	Nej	-	Ja	Allergisk betinget hudbetændelse
GG	-	-	Nej	Ja	Allergi	Ja	Medicin intolerant, aflivet grundet svær allergi

Hund	25: Andre helbredsmæssige problemer	26: Behandling	26 Beh. Mod ichthyosis	27: Ichthyosis' betydning
A	Nej	Nej	Nej	Ingen/lille - 0
B	Nej	Nej	-	Moderat - 5,5
C	Nej	-	-	Ingen/lille - 0,5
D	Sten var ikke faldet ned i pungen. Den ene knogle i forben voksede skævt= operation	Mobility fra equidan	Nej	Moderat - 7
E	Nej	-	-	Ingen/lille - 0
F	Nej	Nej	Nej	Ingen/ lille - 1
G	Nej	-	-	Ingen/lille - 3
H	Nej	-	-	Ingen/lille - 0
I	Nej	Nej	Nej	Moderat - 5
J	Nej	Nej	Nej	Moderat - 7
K	Ja, røde øjne - får øjendråber	-	-	Ingen/lille - 1
L	Svamp i ørerne	Nej	Nej	Ingen/lille - 2,5
M	Ledmus i albueled	Canosan	Nej	Ingen/lille - 1,5
N	Nej	Nej	Nej	Ingen/lille- 0
O	Øremider som hvalp + regnbuehindebetændelse	Nej	Nej	Stor/meget stor - 8
P	Nej	-	Nej	Ingen/lille - 1
Q	Epilepsy	Nej	Nej	-
R	Medfødte gigforandringer i forben + kennel hoste (1 gang)	Glukosamin mod gigt med godt resultat	Nej	Ingen/lille - 1,5
S	TBE, pyometra + tumor på halsen, malign. Fjernet, men recidiv	Ja, rimadyl det sdiste år + canosan	Nej	Ingen/lille - 0
T	Borrelia	Advocate + bayvantic	-	Ingen/lille - 0
U	Nej	Nej	Nej	Ingen/lille - 0
V	Vokseværk + blærebetændelse	Nej	Nej	Moderat - 5
W	Nej	Nej	-	Ingen/lille - 1
X	Nej	Nej	-	Ingen/lille - 0
Y	Vokseværk + tandsten	Nej	-	Ingen/lille - 0
Z	Nej	-	Nej	Stor/meget stor - 8,5
Æ	Nej	-	Nej	Ingen/lille - 0
Ø	Nej	-	-	Ingen/lille - 0
Å	Nej	Nej	-	Ingen/lille - 3
AA	Nej	Nej	Nej	Ingen/lille - 0
BB	Nej	Nej	Nej	Ingen/lille - 0
CC	Nej	Nej	Nej	Ingen/lille - 0
DD	Nej	Nej	Nej	Ingen/lille - 0
EE	Nej	Nej	Nej	Ingen/lille - 0
FF	Nej	Nej	Nej	Stor/meget stor - 10
GG	-	-	-	Stor/meget stor - 8

Hund	27: Tænker over ichthyosis	27: Gør ekstra rent	27: Undlader at tage på besøg	27: Lukker hunden i andet rum	27: Ikke købt hunden	27: Allergirisiko
A	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig
B	Delvis enig	Delvis enig	Helt uenig	Helt uenig	Delvis uenig	Delvis uenig
C	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig
D	Delvis uenig	Hverken/eller	Helt uenig	Helt uenig	Helt uenig	Helt uenig
E	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig
F	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig
G	Hverken/eller	Hverken/eller	Helt uenig	Helt uenig	Delvis enig	Helt enig
H	Helt uenig	Delvis uenig	Helt uenig	Helt uenig	Helt uenig	Delvis uenig
I	Hverken/eller	Helt uenig	Helt uenig	Helt uenig	Delvis uenig	Helt uenig
J	Delvis uenig	Delvis enig	Hverken/eller	Helt uenig	Delvis enig	Helt uenig
K	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig
L	Delvis uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig
M	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig
N	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig
O	Helt enig	Helt enig	Delvis enig	Delvis uenig	Helt enig	Delvis enig
P	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Delvis uenig	Helt uenig
Q	Hverken/eller	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig
R	Helt uenig	Delvis enig	Helt uenig	Helt uenig	Delvis enig	Delvis enig
S	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig
T	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig
U	Helt uenig	Helt enig	Helt uenig	Helt uenig	Helt uenig	Helt uenig
V	Delvis enig	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig
W	Helt uenig	Delvis uenig	Helt uenig	Helt uenig	Hverken/eller	Helt uenig
X	Helt uenig	Helt enig	Helt uenig	Helt uenig	Helt uenig	Helt uenig
Y	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig
Z	Delvis enig	Helt enig	Hverken/eller	Helt uenig	Helt enig	Helt enig
Æ	-	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig
Ø	Helt enig	Delvis enig	Helt uenig	Helt uenig	Helt uenig + hverken/eller	Helt uenig
Å	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig
AA	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig
BB	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig
CC	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig
DD	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig
EE	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Helt uenig
FF	Helt enig	Helt enig	Helt enig	Hverken/ eller	Helt enig	Hverken/ eller
GG	Delvis enig	Helt enig	Delvis enig	Hverken/eller	Hverken/eller	Hverken/eller

Hund	27: Børster oftere	27: Vasker oftere	27: Reduktion i forekomst	27: Omgivelsernes opfattelse	28: Ny hund - Golden igen?	Hvis nej, hvorfor
A	Helt uenig	Helt uenig	-	Delvis enig	Ja, helt sikkert	-
B	Helt uenig	Delvis uenig	Delvis enig	Helt uenig	Ja, højst sandsynligt	-
C	Helt uenig	Helt uenig	Helt enig	Helt uenig	Ja, helt sikkert	-
D	Helt uenig	Helt uenig	Delvis enig	Helt uenig	Ja, højst sandsynligt	-
E	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Ja, helt sikkert	-
F	Helt uenig	Helt uenig	Helt enig	Helt uenig	Ja, højst sandsynligt	-
G	Hverken/eller	Helt uenig	Helt enig	Delvis uenig	Ja, højst sandsynligt	-
H	Helt uenig	Helt uenig	Delvis uenig	Helt uenig	Ja, helt sikkert	-
I	Hverken/eller	Hverken/ eller	Delvis enig	Helt uenig	Ja, helt sikkert	-
J	Helt uenig	Helt enig	Helt enig	Hverken/eller	Ja, helt sikkert	-
K	Helt uenig	Helt uenig	Delvis uenig	Helt uenig	Ja, helt sikkert	-
L	Helt uenig	Helt uenig	Helt enig	Helt uenig	Ja, helt sikkert	-
M	Helt uenig	Helt uenig	Delvis enig	Helt uenig	Ja, højst sandsynligt	-
N	Helt uenig	Helt uenig	Delvis enig	Helt uenig	Ja, helt sikkert	-
O	Delvis enig	Hverken/ eller	Helt enig	Hverken/ eller	Ja, højst sandsynligt	-
P	Helt uenig	Helt uenig	Delvis enig	Helt uenig	Ja, helt sikkert	-
Q	Helt uenig	Helt uenig	Helt enig	Helt uenig	Ja, helt sikkert	-
R	Helt uenig	Helt uenig	Helt enig	Helt uenig	Ja, højst sandsynligt	-
S	Helt uenig	Helt uenig	Delvis uenig	Helt uenig	Ja, helt sikkert	-
T	Helt uenig	Helt uenig	Delvis uenig	Helt ueing	Ja, helt sikkert	-
U	Helt uenig	Helt uenig	Helt enig	Helt uenig	Ja, helt sikkert	-
V	Helt uenig	Helt uenig	Delvis uenig	Helt uenig	Ja, helt sikkert	-
W	Helt uenig	Helt uenig	Delvis enig	Helt uenig	Ja, højst sandsynligt	-
X	Helt uenig	Helt uenig	Delvis enig	Helt uenig	Ja, helt sikkert	-
Y	Helt uenig	Helt uenig	Helt enig	Delvis uenig	Ja, helt sikkert	-
Z	Delvis enig	Helt enig	Helt enig	Delvis enig	Ja, højst sandsynligt	-
Æ	Helt uenig	Helt uenig	Helt uenig	Helt uenig	Ja, helt sikkert	-
Ø	Helt uenig	Helt uenig	Helt enig	Helt uenig	Ja, helt sikkert	-
Å	Helt uenig	Helt uenig	Delvis uenig	Helt uenig	Ja, helt sikkert	-
AA	Helt uenig	Helt uenig	Delvis enig	Helt uenig	Ja, helt sikkert	-
BB	Helt uenig	Helt uenig	Delvis enig	Helt uenig	Ja, helt sikkert	-
CC	Helt uenig	Helt uenig	Delvis enig	Helt uenig	Ja, helt sikkert	-
DD	Helt uenig	Helt uenig	Delvis enig	Helt uenig	Ja, helt sikkert	-
EE	Helt uenig	Helt uenig	Delvis enig	Helt uenig	Ja, helt sikkert	-
FF	Helt enig	Helt enig	Helt enig	Hverken/eller	Ved ikke	-
GG	Delvis enig	Delvis enig	Helt enig	Helt uenig	Nej, helt sikkert ikke	Forløbet har været for hårdt

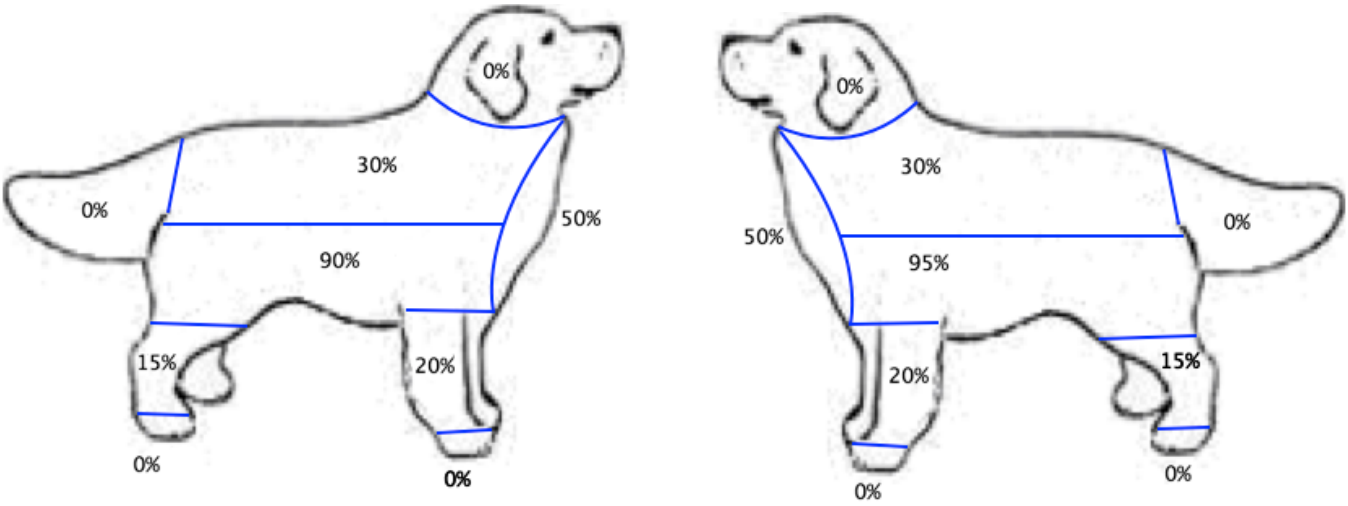
Hund	29: Ny hund med ichthyosis	30: Kosmetisk problem?	30: Graduer problemet	30: Sygdom?	30: Graduer problemet	Indavlsgrad (3 generationer)
A	Ingen betydning	Nej	-	Nej	-	0%
B	Stor betydning	Ja	Moderat	Ja	Moderat	0%
C	Lidt betydning	Ja	Lille	Ja	Lille	0%
D	Lidt betydning	Ja	Stor	Ja	Moderat	0%
E	Ingen betydning	Nej	Lille	Nej	Lille	0%
F	Stor betydning	Ja	Lille	Nej	-	0%
G	Meget stor betydning	Nej	-	Ja	Lille	0%
H	Ingen betydning	Ja	Lille	Nej	-	0%
I	Lidt betydning	Ja	Lille	Nej	-	0%
J	Meget stor betydning	-	-	Ja	Moderat	0%
K	Ingen betydning	Ja	lille	Nej	Lille	0%
L	Lidt betydning	Ja	lille	Nej	-	0%
M	Lidt betydning	Ja	Lille	Nej	Lille	0%
N	Ingen betydning/ Meget stor betydning	Ja	Lille	Nej	-	0%
O	Meget stor betydning	Ja	Stor	Ja	Moderat	0%
P	Lidt betydning	Ja	Stor	Nej	-	0%
Q	Stor betydning	Ja	lille	Ja	Moderat	0%
R	Stor betydning	Nej	-	Ja	Stor	0%
S	Ingen betydning	Ja	Lille - stor	Ja	Lille	12,5%
T	Ingen betydning	Ja	Lille - stor	Ja	Lille	0%
U	Lidt betydning	Ja	Lille	Nej	-	0%
V	Lidt betydning	Ja	Lille	Nej	-	0%
W	Lidt betydning	Ja	Moderat	Nej	-	0%
X	Lidt betydning	Nej	-	Ja	Moderat	0%
Y	Ingen betydning	Ja	Lille	-	-	9,375%
Z	Meget stor betydning	-	-	Ja	Moderat	3,125%
Æ	Ingen betydning	Ja	Lille	Nej	-	0%
Ø	Lidt betydning	Ja	Moderat	Ja/nej	Moderat	0%
Å	Lidt betydning	Ja	Lille	-	-	0%
AA	Ingen betydning	Ja	Lille	Nej	-	0%
BB	Ingen betydning	Ja	Lille	Nej	-	0%
CC	Ingen betydning	Ja	Lille	Nej	-	0%
DD	Ingen betydning	Ja	Lille	Nej	-	0%
EE	Ingen betydning	Ja	Lille	Nej	-	0%
FF	Meget stor betydning	Ja + nej	Stor	Ja	Stor	0%
GG	-	Nej	-	Ja	Stor	0%

Appendix 3 - Distribution of symptoms (question 7 and 13)

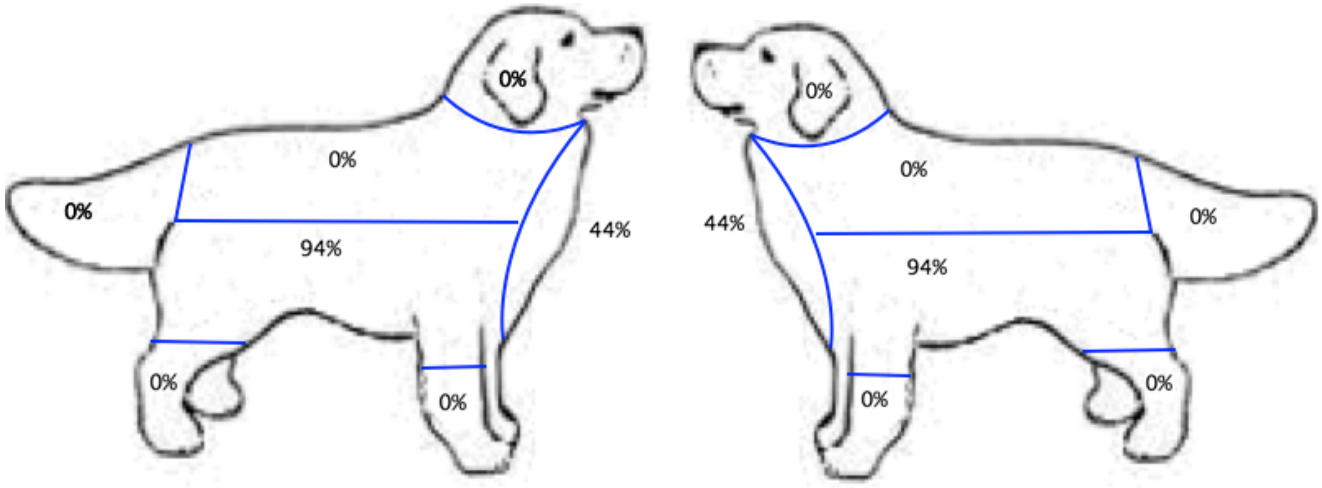
The distribution percentages are based on the number of dogs with the symptom, compared to the total number of dogs.

Example: 6 owners indicated scaling on the dorsal part of the body. The total number of dogs is 20. The percentage is: 6/20*100= 30%

Distribution of scaling: Number of dogs: 20



Distribution of hyperpigmentation: Number of dogs: 16



Appendix 4 – Extra questions

Spørgsmål 1: Har du tidligere opdrættet eller opdrætter du på nuværende tidspunkt Golden retriever hvalpe?
Hvis ja, hvor mange kuld har du fået i alt?

Spørgsmål 2: Spørgsmålet består af to underspørgsmål:

Hvilket af følgende udsagn er du mest enig i? (kun ét svar)

Hvilket af følgende udsagn er du mindst enig i? (kun ét svar)

A: Jeg mener, at de gener og omkostninger ichthyosis har for min(e) hund(e) er for store

B: Jeg mener, at de gener og omkostninger ichthyosis har for mig som ejer er for store

C: Jeg mener, at de gener og omkostninger ichthyosis har for racen som helhed og for racens omdømme er for store

D: Jeg mener, at man bør reducere forekomsten af alle kendte arvelige lidelser uanset, hvilken grad af gene disse måtte fremkalde

Hund	**Spørgsmål 1: Antal kuld**	**Spørgsmål 2: Hvilket udsagn er ejer mest og mindst enig i**	
		Mest	**Mindst**
A			
B			
C			
D	0	D	B
E	-		
F	0	D	A
G			
H	0		
I	20	D	B
J	0	D	A
K	0		
L	0		
M			
N	7		

O	0	D	A
P	14	D	A
Q	-		
R	0	C	B
S			
T	0		
U	0	A	D
V	0		
W	0	A	B
X	0	D	A
Y	6	C	A
Z	0	B	D
Æ	177 hvalpe		
Ø	-		
Å	10		
AA	10	D	A
BB			
CC			
DD			
EE			
FF			
GG	-		