Swedish University of Agricultural Sciences
Faculty of Veterinary Medicine and Animal Science

Review of the literature and an attempt to evaluate intake levels of iodine and selenium in dogs with and without lymphocytic thyroiditis

Janet Johansson

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Department of Clinical Sciences
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Litteraturgenomgång om sköldkörtelrubbning samt ett försök att undersöka jod och selenintag hos hundar med och utan lymfocytär thyroidit

Janet Johansson

Supervisors:
Åke Hedhammar, SLU, Department of Clinical Sciences
Katarina Sundberg, SLU, Department of Animal Breeding and Genetics

Examiner:
Bernt Jones, SLU, Department of Clinical Sciences

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SAMMANFATTNING

Den primära frågeställningen för detta examensarbete var om jod i överskott eller underskott under specifika perioder av en hunds liv kunde påverka huruvida individen skulle utveckla autoimmun sköldkörtelrubbning, lymfocytär thyroidit eller inte. En sekundär frågeställning var om även selen i överskott eller underskott kunde vara en riskfaktor. Ett frågeformulär skickades till hundägare som sedan tidigare ingår i en omfattande studie av genetiska riskfaktorer . Frågorna handlade om vilka foder hunden har ätit under sin livstid (som valp, junior och vuxen). Frågor ställdes även om hundarna fått några tillskott till fodret och i så fall vilka. Arbetet kompletterades med en omfattande litteraturstudie för att förklara hur jod och selen påverkar hormonmetabolismen samt hur den påverkas av överskott respektive underskott. Människor i delar av världen där jodintaget är stort utvecklar många gånger hypothyroidism pga. en autoimmun reaktion som på människa kallas Hashimoto's sjukdom. Motsvarigheten på hund kallas Lymfocytärthyroidit och har många likheter med Hashimoto's på människa. Tidigare studier har indikerat ett möjligt samband mellan ett alltför rikligt intag av jod och utvecklingen av Lymfocytär thyroidit hos hund. Resultatet visade att de flesta hundarna under någon gång i sitt liv äter en diet med högt jodinnhåll. Pga bristande uppgifter om seleninnehåll kunde dess effekter inte utvärderas. Något klart samband mellan jod och utvecklingen av autoimmun sköldkörtelrubbning kunde inte påvisas.

ABSTRACT

The prime aim of this thesis was to investigate whether high or low iodine intake during certain periods of life was, or was not a strong risk factor for the development of canine lymphocytic thyroiditis (CLT). And secondly, if a high or low selenium intake in a combination with high or low iodine intake also may have an influence on that risk factor. To investigate this, a dietary questionnaire was sent out to dog owners. The dogs were selected from an ongoing genetic study of CLT. The questions investigated brand and type of feed during different life stages (puppy, junior and adult) and also if there were any use of dietary supplements. The thesis was also extended with a substantial literature review about the relationship between iodine, selenium (both excess and deficiency) and the thyroid hormone metabolism.

In some parts of the world, humans suffer from an autoimmune thyroid disease called Hashimoto's as a result of high iodine intake. Previous studies have indicated that high iodine intake could also be linked to canine lymphocyticthyroiditis, the dogs equivalent to Hashimoto´s disease. The result of the questionnaire showed that most dogs have had high iodine feeds during some period in life. Due to lack of data regarding selenium content in feeds, these effects could not be evaluated. Any distinct connection between iodine intake and development of canine lymphocytic thyroiditis could not be proven.

Innehåll

INTRODUCTION

Canine lymphocytic thyroiditis (CLT) is an autoimmune endocrine disease, resulting in impaired thyroid function and eventually hypothyroidism. Hashimoto's disease in humans shows many similarities but also some differences compared to CLT in dogs.

Various environmental factors have been shown to have an influence on autoimmune diseases in both humans and animals. For autoimmune thyroid diseases, iodine intake is one of the most well known risk factors. For example, in the autoimmune prone non obese (NOD)-$H2^{h4}$ mouse strain, more than 60 % of the individuals develop hypothyroidism if excessive iodine is added to the drinking water, while only 5 % develop the disease spontaneously with only adequate iodine intake (Rasooly, Burek and Rose 1996).

However, not much research has been performed on iodine requirement and effects of excessive iodine in dogs. To define the minimum iodine limit Belshaw and others performed a study on adult beagles in 1975. Their results indicated140 µg as the lower limit of daily iodine intake. Later, in a study by Castillio et al (2001a), beagle puppies received a diet with high iodine content from 45-90 days of age. The result was decreased plasma free T_4 levels and increased TSH levels, indicative of lymphocytic thyroiditis (Castillio*et al.*, 2001a).

In an unpublished study by Sallander and others (2005), the iodine content of 31 commercially available dog feeds and one algae product on the Swedish market were analyzed. The striking finding was that some feeds contained very high iodine levels and some very low.

Our prime hypothesis for this thesis is that high or low iodine intake during certain periods of life is a strong risk factor for CLT in dogs that is also genetically prone to develop autoimmune thyroiditis. And secondly, that a high or low selenium intake in a combination with high or low iodine intake also may have an influence on that risk factor. To investigate this, a dietary questionnaire was sent out to dog owners. The dogs were selected from an ongoing genetic study of CLT in hovawart and giant schnauzers at the Swedish university of agricultural sciences, Uppsala. The questions investigated brand and type of feed during different life stages as well as use of any dietary supplements.

THE THYROID GLAND AND AUTOIMMUNITY

Function

The thyroid gland is located under the larynx and behind the oesophagus in the throat. The thyroid tissue consists of follicles, in which the hormone synthesis is performed. The follicles have a single layer of epithelial cells and in the centre of the follicles is the colloid. The main function of the gland is to produce thyroid hormones. These hormones have a profound impact on the body's metabolism. The two hormones which is produced in the thyroid are 3,5,3'-tri-idiothyronine (T_3) and 3,5,3',5'-tetraiodothyronine, also called thyroxin (T_4). As the name indicates, T_3 contains three iodine ions and T_4 contains four, see figure 2. The thyroid hormone synthesis starts midway in the development of the foetus (Sjaastad, Hove and Sand 2003) with help from enzymes. The reaction is catalyzed by hydrogenperoxide (H_2O_2) (Artur and Beckett 1999). T_4 is an inactive prohormone, which converts into the second biologically active T_3 when needed in the body tissues, with help from deiodinase (Ruz*et al.*, 1998), see illustration figure 1.

The role of iodine

Iodine is necessary for the thyroid hormone production. Iodide (I^-) is transported in the bloodstream from the gastrointestinal tract to the thyroid, stomach, salivary and lacrimal glands

and the mammary gland (during lactation). The transport of iodide into the thyroid cells occurs by secondary active transport with a sodium iodide symporter (NIS). The iodide transports from the blood against its electrochemical gradient together with sodium ions. I^- is then transported into the thyroidal colloid by pendrin, a transport protein, see figure 1. Thyroid hormone release is regulated by a negative feedback mechanism. Thyroid stimulating hormone (TSH) is released from the pituitary gland and TSH receptors are located on the membrane of thyroid cells (Spitzweg and Morris 2002).

Hormone synthesis

Both T_3 and T_4 are produced in the thyroid and released into the bloodstream. T_4 are then converted into T_3 in target tissues. The thyroid hormones are synthesized by coupling of iodine to thyroglobulin (Tg) to form iodothyrosine, the process is catalyzed by thyroid peroxidase (TPO). Several iodothyrosine residues are then combined to form T_3 and T_4, respectively (see figure 1) (Spitzweg and Morris 2002). Hormone synthesis therefore requires sufficient amounts of iodine and continuous production of hydrogen peroxide (H_2O_2) which is also present in the formation of T_3 and T_4 (Köhrle *et al.*, 2005). Although intracellular H_2O_2 is necessary for thyroid hormone production, high H_2O_2 concentrations can cause toxicity and apoptosis. H_2O_2 increases in the thyroid in case of high levels of plasma TSH (Demelash *et al.*, 2004). H_2O_2 is degraded by glutathione peroxidase (GPX) (Corvilain*et al.*, 1993).

There are several GPX enzymes and the one that is synthesized in the thyroid is called GPX-3. This GPX is also synthesized in several other tissues e.g. kidney and lungs. The function of these GPX enzymes is to protect the cells against damage by free radicals. There are four known GPX which contains selenium, therefore the production of these enzymes requires sufficient amounts of selenium. GPX-1 protects the red blood cells from haemolysis, GPX-2 is distributed in the gastrointestinal tract, GPX-3 protects the thyroid and GPX-4 metabolizes phospholipidhydroperoxidases (Arthur 2000).

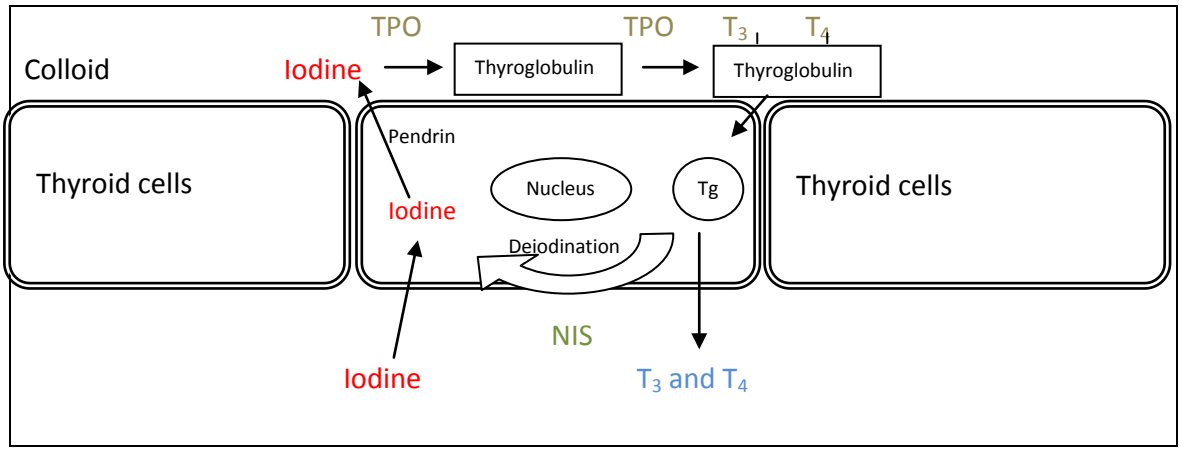

Figure 1. Illustration of hormone synthesis from iodine in the thyroid gland.

The role of deiodinase and selenium

Deiodinase is a peroxidase enzyme that is involved in the activation or deactivation of thyroid hormones. There are three types of deiodinases; type I (5'D1), II (5'D2) and III (5D3). Type I deiodinase is needed when T_4 converts into T_3 and decouples the iodine from the prohormone, and is mainly located in the liver, kidneys, thyroid and in peripheral tissues. Type II is mainly located

in the brain, pituitary, brown adipose tissue and placenta. Type III is located in the brain. Selenium deficiency decreases the type I and type II enzyme activity (Köhrle *et al.*, 2005).

Type I 5'-deiodinase (5'D1) catalyses the degradation of T_4 to T_3 by removing an iodide ion from the 5(3) position of the thyrosyl ring, see figure 2. 5'D1 is expressed in the liver, kidney, thyroid and pituitary. Most of the circulating T_3 is assumed to be produced in the liver and thyroid by 5'D1 (Köhrle *et al.*,2005). Selenium deficiency reduces 5'D1 activity and repletion increases it but in case of severe selenium deficiency, selenium is reorganized in different organs. Selenium concentration in liver, kidney, heart, skin and muscles are decreased while in the thyroid, other endocrine organs, reproductive system and the brain the levels of selenium are maintained. The 5'D1 enzyme activity are thereby kept at a high level. A lower activity of 5'D1 in liver and kidney contributes to a higher plasma total and free T_4 concentrations (Bermando *et al.*, 1995).
Type II 5'deiodinase (5'D2) also catalyses the degradation of T_4 to T_3. But unlike 5'D1, 5'D2 produces T_3 for intracellular needs and is not contributing to circulating T_3 and is regulated by T_4 (Köhrle *et al.*, 2005).
Type III 5-deiodonase (5D3) inactivates T_3 and T_4 by degeneration to T_0, which lacks thyromimetic activity. High levels of 5D3 are found in many tissues including the brain and skin but not in liver or kidneys (Köhrle*et al.*, 2005).

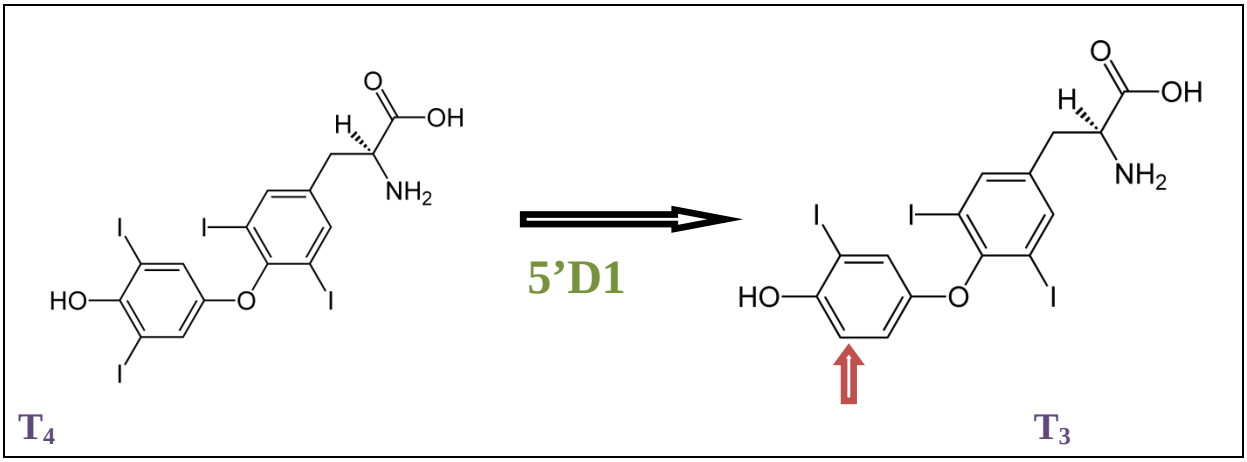

Figure 2. Deiodination of T_4 into T_3. The small arrow points to the position where the deiodinase have disconnected the iodine.

Development of autoimmunethyroiditis

Autoimmunity

In an autoimmune disease, the immune system is not able to distinguish between "self" and "non-self" and may therefore target an immune reaction with T cells towards its own organ or tissue (Sjaastad, Hove and Sand 2003). The development of autoimmune thyroiditis starts in the thyroid gland and is divided into three phases.

First phase

In a first afferent phase antigen presenting cells (APC), monocytes accumulates in the gland and attach with relevant autoantigens.

Second phase

In the second central phase, APC seeks contact with T cells and B cells in the lymph nodes. As a result an immune response is triggered and this generates an autoimmune reaction. T cells then

attacks target cells and more antigens are released. When the antigens are released, the B cells produce autoantibodies.

Third phase

In the last effector phase auto-reactive T cells and B cells gain access to target glands. APC then changes to T helper cells and the inflammation accumulate. Macrophages are then activated to kill the target cells. After the third phase, the thyroid gradually dissolves (Lam-Tse *et al.,* 2002).

Hypothyroidism

In hypothyroidism the production of thyroid hormones is impaired. The symptoms are intolerance to cold, depression, unable to concentrate and muscle weakness (Sand *et al.,* 2006).

Hypothyroidism due to iodine deficiency is most common in severely iodine deficient areas. Iodine deficiency often causes an enlargement of the thyroid gland (goiter) (Sjaastad, Hove and Sand 2003).

Hyperthyroidism

In hyperthyroidism there is instead an overproduction of thyroid hormones and also an enlargement of the thyroid gland. People with hyperthyroidism are treated with radioactive iodine resulting in destruction in parts of the thyroid or surgical removal of parts of the thyroid (Sjaastad, Hove and Sand 2003).

Autoimmune thyroiditis in humans

Hashimoto's disease

In non-iodine deficient areas Hashimoto's disease is the most common cause of hypothyroidism.
Hashimoto's is an autoimmune disorder. It is characterized by gradual loss of thyroid function and infiltration of T-cells. Patients with Hashimoto's disease develop TPO autoantibodies and sometimes also Tg-autoantibodies. The symptoms are weight gain, intolerance to cold, dry skin, depression and growth failure. Patients have high levels of TSH and low levels of T_3 and T_4 (Michels and Eisenbarth 2010).

Graves' disease

Hyperthyroidism is most commonly caused by an autoimmune reaction named Graves' disease and results in an increased body metabolism. The symptoms are increased heart rate, weight loss, sweating and irregular heartbeat (Sand *et al.,* 2006).
Grave' disease is more common in areas with moderate to high intake of iodine. Autoantibodies stimulate the thyroid gland receptors, causing high production of thyroid hormones (Umar*et al.,* 2010).

Hypothyroidism in dogs

The most common cause of low or no production of thyroid hormones in dogs is autoimmune destruction of the thyroid by lymphocytic thyroiditis (Sjaastad, Hove and Sand 2003).

Autoimmune thyroiditis in dogs

Canine Lymphoytic thyroiditis is characterized by destruction of the thyroid by autoantibodies and infiltration of lymphocytes. As the thyroid degenerates the gland produces less thyroid

hormones and hypothyroidism is a result of the progression. The symptoms are diffuse and the development of lymphocytic thyroiditis is sometimes slow. As a consequence, it could be difficult to reduce the incidence of lymphocytic thyroiditis by traditional breeding strategies, since affected dogs may have been used in breeding at the time of diagnose. Another issue in breeding is that lymphocytic thyroiditis is a complex disease affected by several genes and environmental factors. The disease cannot be cured and affected dogs are therefore dependent on lifelong medication with synthetic thyroxin.

According to the insurance statistics, hypothyroidism is the most common endocrine disease in dogs today (Egenvall *et al.,* 2000). The most common symptoms are obesity, seborrhoea, alopecia, weakness, lethargy, bradycardia (lowered heart rate) and pyoderma (Panciera 1994). The disease is diagnosed by measurement of free T_4, TSH and thyroglobulinautoantobodies (TgAA) in plasma. In a Swedish study based on insurance statistics of the entire dog population and not just affected dogs, the most affected breeds were Giant Schnauzer and Howavarts (Egenvall *et al., 2000*).

Hyperthyroidism is extremely rare in dogs.

DEVELOPMENT OF AUTOIMMUNE THYROIDITIS BY GENES AND ENVIRONMENTAL INTERACTIONS

The environmental factor in development of thyroiditis in a nonobese diabetic (NOD)-$H2^{h4}$ mouse strain is quite obvious. Only a few percent of the (NOD)-$H2^{h4}$ mouse strain develops autoimmune thyroiditis spontaneously. But, when these animals were exposed to an increased iodine intake (0,05% iodine in drinking water), as much as 60 percent of the individuals developed thyroiditis after 8 weeks of high iodine intake. This indicates a genetic predisposition for the disease within the strain, but also that high iodine intake in autoimmune-prone animals leads to a higher prevalence of autoimmune thyroiditis compared to non prone individuals (Rasooly *et al.,* 1996). In a comparison with non prone rat strains high iodine intake instead decreased the incidence of thyroiditis (Ruwhof and Drexhage 2001).

The genetic background to autoimmune thyroiditis in humans

The genetic make-up is an important risk factor in autoimmune thyroiditis. In humans, high iodine intake in autoimmune prone individuals could accelerate autoimmune thyroiditis, while in non-autoimmune prone this only causes temporary disturbances in the thyroid function (Ruwhof and Drexhage 2001).

HLA-DR, CTLA-4, CD40, PTPN-22 and the Tg-gene are all genes that have been associated with autoimmune thyroid disease (AITD) in humans (Tomer and Huber 2009). Graves' disease and Hashimoto's disease are both autoimmune thyroid diseases in humans. Hashimoto's disease has many similarities with lymphocytic thyroiditis in dogs. Hashimoto's is characterized by hypothyroidism and presence of autoantibodies against thyroglobulin and thyroperoxidase. Graves' disease patients develop autoantibodies against TSH-receptors and the result is hyperthyroidism (Jacobson and Tomer 2007). The concordance rate for autoimmune thyroiditisin monozygotic twins is below 100% which also indicates that environmental factors have an impact on the development of the disease (Rose*et al.,* 1999).

In humans, females have a higher prevalence of Hashimoto's thyroiditis. The occurrence of autoantibodies is also related to iodine intake. A higher occurrence of thyroglobulinautoantibodies (21 %) was found in people living in mild iodine deficient areas compared to areas with sufficient iodine intake (10%). Prevalence of hypothyroidism was also higher in iodine sufficient areas (Andersen *et al.*, 2009).

Environmental factors affecting autoimmune thyroiditis in man

There are several studies investigating environmental factors of importance in the complex picture of autoimmune thyroid diseases in humans. Factors that increase the risk of autoimmune thyroid diseases are low birth weight, high and low iodine intake, low selenium intake, stress, allergy, smoking and infections (Prummel *et al.*, 2004). There is a large variation in iodine intake around the world. For example, Icelandic people have a high iodine intake and Danish people principally have low iodine intake. Furthermore disease prevalence varies between these two populations. A comparative study of Iceland versus Denmark indicated a higher prevalence of hyperthyroidism in Iceland, while hypothyroidism was more common in Denmark. One proposed explanation is the differences in iodine intake between these countries (Laurberg *et al.*, 1998).

Genetic studies on lymphocytic thyroiditis in dogs

In contrast to Hashimoto´s disease in humans, not much genetic research has been done on canine lymphocytic thyroiditis. Conway et al studied the disease in a family of Borzoi dogs back in 1985 and concluded that the disease is indeed hereditary and proposed an autosomal recessive mode of inheritance (Conway *et al.*, 1985). However, today it is commonly accepted that disease development involves not only one, but several genes and also environmental factors (Ruwhof and Drexhage 2001). The only genetic risk factor associated to canine lymphocytic thyroiditis at the moment is the dog leukocyte antigen class II genes, which are the dog equivalences to human leukocyte antigen (HLA) class II genes. In a study of 74 cases and 30 healthy controls sequencing of the polymorphic exon 2 of DLA-DRB1, -DQA1 and -DQB1 revealed a risk haplotype (OR 6.5) as well as a protective haplotype (OR 0.3) (Wilbe *et al.*, 2010). Although DLA-genotype has been shown to contribute to the disease, it is not solely responsible for disease development but rather act as one of several collaborative genetic risk factors. In order to identify all the major genetic risk factors for the disease, a large research effort has been ongoing at the Swedish university of agricultural sciences since 2005. A genome wide association analysis of 118 giant schnauzers (69 cases and 49 controls) using 50 000 SNPs has identified 7 large loci of association (best p-value: 10^{-6}), the majority harbouring genes with known immune regulating function (Sundberg *et al.*, in prep). Next step involves identification of the exact mutations and development of an effective breeding tool, which in a long-term perspective could be used to greatly reduce disease prevalence. The final goal is to indicate interactions between genetic and environmental risk factors

Why use the dog as a model for complex diseases?

Today the dog is used as a model in search for mutations causing genetic diseases in human. Dogs tend to develop the same genetic diseases as humans, for example cancer, diabetes or epilepsy. They also live in the same environment as we do. The dog has undergone several genetic bottle necks during the evolutionary progress from wolf several thousand years ago to today's domestic dog. Today there are several hundred breeds, all with distinguish character and exterior. Some

breeds have higher prevalence of specific diseases, which make them good candidates in the finding of disease-causing genes (Karlsson and Lindblad-Toh 2008).
The first step is the genome-wide association analysis, where you search the entire genome for association between genetic markers (SNPs) and a specific phenotype. In this stage you compare the genetic makeup of healthy versus unhealthy dogs (e.g. hypothyroid) within the same breed. The hypothesis of this method is that individuals from the same breed have many gene variants in common, but the unhealthy group differ from the healthy in gene regions that influence the disease they are suffering from (see figure 3). In the second stage, called fine-mapping, you take advantage of the fact that individuals from different dog breeds have less gene variants in common. You now concentrate on the associated region found in stage one, and compare the genetic make up of unhealthy (e.g hypothyroid) dogs from several different breeds. By doing this, the associated region can be narrowed down, revealing one or a few candidate genes (figure 3) (Karlsson and Lindblad-Toh 2008). Ongoing research at the Swedish university of agricultural sciences aim to identify genes responsible for lymphocytic thyroiditis in dogs. The genome wide association was made in two separate breeds; the hovawart and the giant schnauzer, while fine-mapping was done in several breeds (Sundberg *et al.*, in prep).
In complex diseases like CLT, spontaneously occurring cases can be investigated and compared to healthy dogs not only for genetic variations but also environmental exposure.

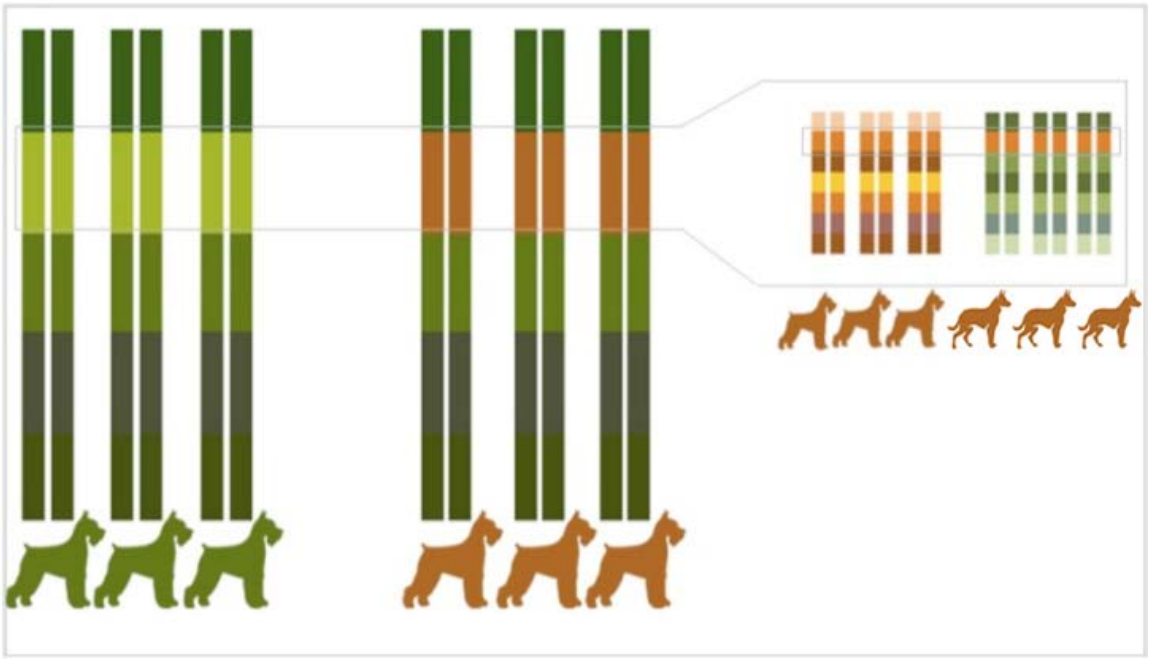

Figure 3. Illustration of GWA and fine-mapping. In step one (GWA), a large genetic region is identified by association mapping between cases and controls of the same breed. In step 2 (fine mapping), cases from several breeds are used and the region can be narrowed down. Green dogs illustrate controls and red illustrate cases.

Environmental factors in dogs

Among the environmental factors seen in human Hashimoto's disease, the dietary factors are probably most likely to affect also dogs. Both too low and too high iodine and selenium intake in different stages of life could be of importance in the development of canine autoimmune thyroiditis.

IODINE, SELENIUM AND THYROID FUNCTION

Thyroid hormone production is dependent on sufficient iodine intake, therefore adequate iodine ingestion is of great importance. Hydrogen peroxide is necessary in the process of hormone production. However, if the hormone production for some reason is too high or if the level of glutathione peroxidase is too low, hydrogen peroxide could be toxic to the cell. This could be the case in severe selenium deficiency.

Iodine intake in humans

Iodine metabolism

About 90 % of absorbed iodine is excreted through the kidneys (Laurberg *et al.,* 2001). Dietary iodine is absorbed and transported as iodide in the blood and is transported into the thyroid via the sodium iodide symporter. Redundant and used iodide is excreted with the urine within 24 hours. Iodide from degraded thyroid hormones is also excreted through the kidneys (Vejbjerg *et al.,* 2009a).

Deficiency and excess of iodine

Abnormal thyroid function has been shown in humans both in areas with iodine deficiency and excess (Laurberg *et al.,* 1998). In areas with iodine deficiency a high prevalence of goitre as well as hyperthyroidism was shown (Laurberg *et al.,*1998; Laurberg *et al.,* 2001; Teng *et al.,* 2006) while an increased prevalence of hypothyroidism was observed in areas with excessive iodine intake (Andersen *et al.,* 2009; Laurberg *et al.,* 2001; Ruwhof and Drexhage 2001; Teng *et al.,* 2006; Teng*et al.,* 2008). High intake of iodine may induce autoimmune thyroiditis in humans (Gosselin *et al.,* 1978), and inhibit the iodine metabolism in the thyroid gland by negative feedback (Van Sande *et al.,* 1985).

Measurement of iodine status

In many parts of the world, humans are suffering from iodine deficiency. Iodine status is commonly measured by urinary iodine (UI). There are several methods in measuring UI, such as 24-hour collection, single spot urinary samples, µg iodine/g creatinine and estimated 24-hour iodine (Vejbjerg *et al.,* 2009a).

Measurement of serum thyroblobulin (Tg) is also a way of measuring iodine levels but this method is only used in iodine deficient areas and it is not clear how Tg levels are influenced by high iodine intake (Vejbjerg *et al.,* 2009b).

Iodine requirement in humans

The recommended iodine intake for adult humans is approximately 100 µg/ day (Delange 1994). An intake of 250 µg/ day in mild deficient areas for four months had a great impact on thyroid function. The symptoms were changes in thyroid function and development of subclinical hypothyroidism, a higher iodine secretion in urine was also measured (Reinheart *et al.,* 1998). An even higher intake of iodine (750 µg/ day) resulted in low levels of free T_4 and an increase of TSH (Chow *et al.,* 1991).

Sources of iodine

The iodine content in feedstuff is depending on the iodine content in the soil and water (World health organization, 2010). A variation between locations in metal concentration has been seen in seaweed, but no fluctuations have been measured between seasons (Morrison *et al.*, 2008). Fluctuations in iodine content have been seen in infant formulas (Pedersen *et al.*, 1999), dog feed (Belshaw *et al.*,1975), kelp (Morrison *et al.*, 2008) and vegetables (Wenlock *et al.*, 1982).

Drinking water

Water contains various amounts of iodine (Pedersen *et al.*, 1999; Yang *et al.*, 2007). A study of iodine content in drinking water in 55 Danish locations showed a variation from 1 to 139 µg I/L (Pedersen *et al.*, 1999). The iodine content in drinking water in Huanghua, a community in China varied from 96 to 228 µg/L (Yang *et al.*, 2007).

Meat and dairy products

Milk and dairy products are rich sources of iodine. The feed of dairy cows is enriched with iodine in some parts of the world, especially during the winter (Laurberg and Nöhr 2002; Dahl *et al.*, 2004; Prummel *et al.*, 2004). Fish and fish products also have high iodine content (Wenlock *et al.*, 1982). Fishmeal is used as a source of protein to cattle in Iceland and therefore the Icelandic dairy- and meat products have higher iodine content than meat and by-products from other parts of the world (Laurberg *et al.*, 2001).

Iodine intake in dogs

The recommended allowance of iodine in adult dogs is 880 µg/ kg DM or 29.6 µg/kg $BW^{0,75}$ (Nutrient Requirements of dogs and cats 2003). Beagle is commonly used for experimental studies in dogs and an adult Beagle weights approximately 9-15 kg (Belshaw *et al.*, 1975). The daily-recommended allowance of iodine for a dog with a bodyweight of 12 kg would be 29.6 x $12^{0.75}$ = 190.8 µg/ day (Nutrient Requirements of dogs and cats 2003).

In a published study adult Beagles received 20, 50, 90, 140, 480 µg of iodine respectively, per day. The minimum daily intake of iodine was suggested to be 140 µg/ day. With an intake of 480 µg/day no changes in circulating T_3 and T_4 concentrations were seen, neither any histological changes on the thyroid (Belshaw *et al.*, 1975). The minimum nutrient level of iodine in a diet for adult dogs was set to 0.07 mg/ 400 kcal (Noel och Wills 1996).

Beagle puppies were fed diets with 5.6 mg iodine/kg dry matter from 45 days of age (weaning) to 90 days and resulted in higher TSH values and lower free T_4. A high intake of iodine also increased the iodine:creatinine ratio urinary secretion. The uptake of iodine was measured pre and post intake of high iodine content feed and was significantly lower post intake (Castillio *et al.*, 2001a). Diets with high iodine content also resulted in bone changes in beagle puppies (Castillio *et al.*, 2001b).

Content of Iodine in Algae

Ascophyllum nodosum is an algae used as an additive to dogs. *A. nodosum* belongs to the brown algae's (Phaneuf *et al.*, 1999). Analysis of eight algae (brown, red and green algae) in St. Lawrence River (North America and Canada) showed average iodine content of 22.7 to 763 µg/g dry weight. There were large variations over season and between locations. The algae *Laminaria longicruris* had the highest iodine content and was measured to 2862 µg/g. In *A. nodosum* the iodine content was 758 µg/g ± 168 and in average 482 µg/g (Phaneuf *et al.*, 1999).

Selenium

Selenium is a micro mineral (trace mineral) that is an important component in some proteins referred to as selenoproteins. There are 35 selenoproteins in the body and most of them are enzymes. Important selenoenzymes for thyroid function are glutathione peroxidase (GPX) and deiodinase. It is therefore important with adequate intake of selenium. It has also been shown that excess intake of selenium has disadvantages, the symptoms for excess are vomiting, spasms, staggered gait, salivation, decreased appetite, dyspnea, oral malodor and nail loss (Hand *et al.,* 2000). Selenium is important for T-lymphocytes, therefore selenium deficiency could cause disturbances in the immune system (Prummel *et al.,* 2004). Symptoms of selenium deficiency are muscular dystrophy, reproductive failure, decreased feed intake, subcutaneous oedema and renal mineralization (Hand *et al.,* 2000). During selenium deficiency H_2O_2 breakdown is decreased because of lowered function of the protecting and degrading GPX. But during iodine deficiency GPX increases because of increased levels of TSH (Köhrle *et al.,* 2005). Selenium deficiency causes redistribution of selenium in tissues. Selenium is transported from liver, muscle, skin and other large tissues to the brain, the endocrine organs and the reproductive organs (Behne *et al.,* 1988). In Europe most soils are selenium deficient because selenium occurs in the surface soils. After years of depletion most of the selenium has been washed out (Zimmermann and Köhrle 2002).

Sources of selenium

Selenium content in vegetables and fruit is low. In cereals it varies due to in what part of the world the crop has been grown. Denmark, Finland, New Zeeland, some parts of Russia and China have poor selenium content in their soils while parts of USA, Canada, some parts of China and Ireland has soils with high selenium content. The selenium content of animal by-products varies depending on the selenium content of the feed and soils. Beef, white bread, pork, chicken and eggs contribute to 50% of total selenium in humans in USA (Combs Jr 2001).

Selenium intake in humans

There has not been much research done regarding selenium requirement in humans. The limit for obtaining normal GPX activity in a man with a body weight of 60 kg required an intake of 40 µg of selenium per day (Levander 1991).

In humans with autoimmune thyroiditis an addition of 200 µg Selenomethionine per day for six months resulted in a decrease in TPOAb, but no change was seen in TgAA (Duntas *et al.,* 2003). A result of high selenium intake is also an increase in deiodinase activity in the thyroid (Zimmermann and Köhrle 2002).

Selenium deficiency in rats

The effect of selenium deficient diet for five weeks in rats was an 70-80% increased plasma T_4 concentration, increased plasma T_3 concentration as well as inhibition of 5'D1 in liver and kidney (Beckett *et al.,* 1989).

In rats low selenium diet caused lower plasma and erythrocyte GPX activity (Ruz*et al.,* 1998; Contempré *et al.,* 1996; Behne*et al.,* 1992).

Selenium deficiency in combination with high iodine intake increases the infiltration of mononuclear inflammatory cells and necrosis in the thyroid. However, high iodine in combination

with adequate selenium intake did not cause any destructive process in rat's thyroid (Contempré *et al.*, 1993).

Selenium deficiency in cats

Kittens with selenium deficiency got reduced plasma selenium and GPX activity. Total plasma T_4 increased and total plasma T_3 decreased. This is explained by the fact that the diodinase that converts T_4 to T_3 in tissues is selenium dependent. So as a consequence to lowered plasma selenium concentrations, less T_4 can be converted to T_3 (Yu *et al.*, 2002).

Selenium requirement in dogs

There are no data on either the need for selenium in adult dogs or acceptable safe upper limit. Therefore the recommended allowance for adult dogs, 11.8 µg/ kg $BW^{0.75}$ is only a suggestion based on sufficient intake of growing puppies (Nutrient Requirements of dogs and cats 2003).

Selenium excess in other species

High selenium intake increases the GPX activity in the thyroid in pigs (Zhou *et al.*, 2009), the opposite was seen in rats with decreased GPX activity in the liver but not in plasma. The decreased GPX activity in the liver suggested to be due to other components in the feed, the length of feeding period or the developmental stage of the animal (Behne *et al.*, 1992).

DIETARY SURVEY

QUESTIONNARIRE ABOUT FEEDS AND SUPPLEMENTS

The purpose of this study was to investigate iodine- and selenium intake as environmental factors influencing the development of lymphocytic thyroiditis in two high-risk breeds. Through dog owner questionnaires we aimed to estimate iodine and selenium intake in a group of family dogs and compare it between cases and healthy controls.

MATERIAL AND METHODS

The dog material for the current study is part of a bigger cohort of in total 530 giant schnauzers and 155 hovawarts collected at the Swedish university of agricultural sciences, Uppsala. Blood and sera were collected from each dog and owners were asked to fill in a questionnaire about current diseases, feeding and medication. In order to identify dogs with lymphocytic thyroiditis all serum samples were screened for TgAA-, TSH- and free T_4 levels. The criteria for cases were TSH ≥40 µU/ml and/orTgAA positive and for controls: TSH ≤ 25 µU/ml, TgAA negative, FT_4 5-25 pmol/l and an age of seven years or more.
From this original cohort a subset of 89 giant schnauzers (67 cases and 22 controls) and 32 hovawarts (21 cases and 11 controls) was selected for the current study. Dogs were selected based on disease status (case/control) and current age (≤ 12 years).

A questionnaire (appendix 1) surveying thyroiditis status, type and brand of feed as well as type and amount of supplements through the dogs different life stages (puppy, junior, adult and current) were sent out to the dog owners in three different dispatches. A reminder was sent by e-mail to those with known e-mail addresses. Phone calls were made to those who had not answered

by mail. The dog owners could choose between two options to answer the survey, either by mail or on the Internet.

The first dispatch of dogs investigated consisted of 20 cases and 20 controls born in 2000 or later. The second sent out consisted of 51 cases and 3 controls also born in 2000 or later. The third sent out consisted of 17 cases and 10 controls born in 1998 and 1999. In total 121surveys was sent out (88 cases and 33 controls) (see Table 1). Nine surveys were returned by the post office because of wrong addresses (7 giant schnauzer all cases, as well as one case and one control of hovawarts).

The feeding periods were defined by three age categories: puppy (2-6 month), junior (7-24 month) and adult (>2 years of age). In total, 255 commercial products from 30 different brands were listed. The owners were asked to note what product and possible supplements the dogs were fed in each age category.

Thirty-one commercial products and one supplement had been analyzed for iodine content in a prior, unpublished study (Sallander *et al.*, in prep).

Table 1. *Number of cases and controls and the breeds in the dispatches*

No.		Breed
1.	20 cases	10 Giantschnauzer
		10 Hovawart
	20 controls	14 Giantschnauzer
		6 Hovawart
2.	51 cases	46 Giantschnauzer
		5 Hovawart
	3 controls	3 Giantschnauzer
		0 Hovawart

The result from all surveys was plotted into a excel sheet. The products were categorized according to the iodine content; as low, medium or high, see table 2. The low, medium and high category was set according to NRC (Nutrient Requirements of dogs and cats 2003).

If several feeds within the same brand had been analyzed, a mean value was calculated and used to all feeds within that brand.

Table 2. *Limits for Categoriesof iodine content.*

Category	Amount of iodine mg/kg DM
Low	0-0.7
Medium	0.8-3.9
High	4.0-

RESULT

Excluded surveys

In total 91 surveys were filled in by the dog owners, which gave an answering frequency of 75%. Three controls were dismissed, two because the dog owners declared that their dog was put on medication for lymphocytic thyroiditis and the third because the dog owner said that the dog had been diagnosed with thyroiditis but was now healthy again. One survey sent in on-line was excluded because no dog matched the registration number stated on the survey. Of the remaining 87 surveys, 64 was cases i.e. 73.6% and 23 was controls i.e. 26.4%, see table 3.

Table 3.*Answering frequency.*

No.		Breed	Answers	Frequency	Total	
1.	20 cases	10 Giantschnauzer	9	19/20 = 95%	37/40 = 93%	87/121 = 72%
		10 Hovawart	10			
	20 controls	14 Giantschnauzer	12	18/20 = 90%		
		6 Hovawart	6			
2.	51 cases	46 Giantschnauzer	33	35/51 = 69%	37/54 = 67%	
		5 Hovawart	2			
	3 controls	3 Giantschnauzer	2	2/3 = 67%		
		0 Hovawart	0			
3.	17 cases	11 Giantschnauzer	5	9/17 = 53%	13/27 = 48%	
		6 Hovawart	4			
	10 controls	5 Giantschnauzer	2	4/10 = 40%		
		5 Hovawart	2			

Answering frequency within cases, controls and breeds

Some of the dog owners declared that they did not remember what type of feed they had given their dog in the different age categories. Moreover, some brands of feeds mentioned in the questionnaires had unknown (not analyzed) iodine content. Both those that did not remember and those that had given feeds with unknown iodine content were equally distributed between cases and controls. Also dogs that had been eaten brands of feeds that were analyzed were evenly distributed between cases and controls.

No dog in this study had been fed feeds with low iodine content during all three age categories (puppy, junior and adult).

In total 64.6% of cases and 60.7 % of controls have had at least one feed with high iodine content during some age period in life. 7.7 % of the cases and 4.3% of the controls have never had a feed with high iodine content.

Regarding the Iodine content in diets, a larger part of the puppies had been eaten feeds with high iodine content (66%) compared to junior (56%) and adult (46%).

Supplements

Forty percent reported that they had given some form of supplements, but only 9 % of the dog owners had given algae products to their dog. The distribution of cases and controls among these were 87.5% (7) cases and 12.5% (1) controls. Of the seven cases, three have had at least one high iodine feed during puppy, junior and as adult and one as puppy and junior. The control dog also had at least one high iodine feed fed during puppy, junior and as adult, see table 4.

Table 4. *Iodine content in diets for cases and control that had been given algae supplements.*

	1	2	3	4	5	6	7	8
Puppy	H	H	?	?	H+M	L	NA	H
Junior	H+M	H	NA	?	H+M	H	NA	H
Adult	H+M	H+M	NA	M	M	H	NA	H+M
Case/control	Case	Case	Case	Case	Case	Case	Case	Control

H= high iodine content in feed, M = medium iodine content in feed, L = low iodine feed, ? = the owner does not remember the brand of the feed and NA = the brand was not analyzed in previous study.

All dogs that have had algae products were adult when supplemented, except no 4 that have had algae as a junior.

Oils, vitamins, glucosamine, carrots and peat were the other main categories of supplements given, except algae products. The category of oils included salmon oil, linseed oil, wheat germ oil, fish oil (not specified), rape seed oil, Viacutan®, sunflower oil, omega-3 oil and olive oil. These categories of supplements except algae products and oils all had an even distribution between cases and controls

DISCUSSION

The recommended minimum daily requirement of iodine is 23.6 µg/ kg $BW^{0.75}$ (Nutrient Requirements of dogs and cats 2003). The daily minimum intake for a dog with a bodyweight of 12 kg is thus 23.6 x $12^{0.75}$ = 152.2 µg/ day. This is comparable with a minimum intake for an adult dog in Beagle size of 140 µg/ day.

The recommended upper limit in dog feed is set to 4 mg iodine/kg DM (Nutrient Requirements of dogs and cats 2003). In the analysis of the 31 dog feed, the highest iodine content was 7.03 mg/kg DM which is far over the recommendations by the NRC.

In a study of beagle puppies on a high iodine diet (Castillio *et al.*,2001a), there was no information of the weight of the puppies nor the amount of feed that each puppy was fed each day. The three diets contained of a home prepared diet, which was not analyzed in iodine content, a home prepared diet added with 5.6 mg KI/kg DM but not analyzed and a commercial diet. It is unclear if the commercial diet contains 5.6 mg KI or I/kg DM (Castillio *et al.,* 2001a). Lack of any follow up of these studies indicates that more research is necessary to determine the exact safe upper limit for iodine for growing as well as adult dogs.

Another difficulty is the variation of iodine content in the ingredients of dog feeds. Belshaw et al (1975) noticed that iodine content in the same brand of dog feed fluctuated more than seven-fold between the years 1969 to 1975. Fluctuations have also been seen in different feedstuffs like cereals, meat, fish and fats in Britain during 1977-1979 (Wenlock *et al.*, 1982). Knowing this, we should have in mind that the dog feed analysis used in this study was made only once (2005) which consequently introduces a fair amount of uncertainty. Moreover, the dog owners were asked to fill in what they had fed their dog up to twelve years ago. A dog feed that was analyzed with high iodine content in 2005 would not necessary have high iodine content 1998 or 2010. It would be of great value if the feed companies could declare analysis of both selenium and iodine and made it available for the consumers.

In this thesis it is indicated that additives with algae have high iodine content. It would be interesting to look at the iodine content also in salmon oil and fish oil as well as in the glucosamine products. These products often contain green-lipped mussel and other shellfish, which is rich in iodine.

There are mainly two algae products on the Swedish market. One of them is sold as a mineral additive and the other is sold as a product for dental plaque. Both contain the same algae specie *A. Nodosum*. On the can of the plaque product it is said that the product contains 0.2 mg iodine/g. A dog with a body weight of 12 kg should according to the manufacturer be fed 0.33-0.67 grams per day. If the iodine content is 0.2 mg/g the daily intake from the additive is 66-134 µg iodine. Compared with the NRC recommendations, this additive would not cause iodine excess itself or in combination with a low iodine feed, if the declared iodine content is correct in the plaque product.

However, in the study made by Phanehuf and others in 1999, the mean iodine content of *A. Nodosum* were 482 µg/g. This is more than twice the amount that the plaque product declares. If this is the case, the daily intake of iodine in a dog with a body weight of 12 kg would be between 250 and 3047 µg.

The manufacturer of the mineral additive claims that the iodine content of their product is 500 µg/g. This is quite similar to the content declared by Phanehuf and others (1999). In this product it is recommended to give a dog 1 ml/ 2 kg BW. A dog with a body weight of 12 kg would, according to this have an intake of 2010 µg/day of iodine, assumed that these two algae products have the same weight g/ml. An intake of 2010 µg of iodine is far beyond the recommended allowance of iodine that is 180 µg/day according to the NRC (2003) and there would be a risk of iodine excess.

The energy requirement of an adult dog is 130 kcal/kg $BW^{0,75}$ (Nutrient Requirements of dogs and cats 2003). A dog with a body weight of 12 kg would therefore require 838.16 kcal/ day. The highest iodine content of the analyzed feeds (Sallander on prep) was 7.03 mg/ kg DM. The intake of iodine in an dog with a 12 kg BW would be 1687 µg/day, calculated from the energy content of the feed and the energy requirement according to the NRC (2003).

The only safe upper limit in iodine intake for adult dogs, according to the NRC (2003) is 4 mg/kg DM. Assumed that a dog is fed a high iodine feed and also is fed an algae supplement, the daily

intake of iodine could be as high as 4734 µg and this is far above the recommended daily intake (Nutrient Requirements of dogs and cats 2003).

Selenium

Unfortunately only nine of the 31 feeds were analyzed for selenium with a range from 4.3E-07 to 0.5 mg/kg DM. According to the NRC the adequate intake for an adult dog is 350 µg/kg DM but there is no minimum or upper limit. Therefore, it is very difficult to say whether there is a problem with selenium excess or deficiency.

Apparently the iodine- and maybe also selenium content of dog feeds on the Swedish market varies a lot.

CONCLUSION

More than 60 % of both cases (65%) and controls (61%) in this study have had a high iodine feed intake during some period in life.
Nine % of the dogs had been given algae products with a high Iodine content , out of which 7 were cases and only one had a control status. Many of these dogs were also on diets with a high Iodine content.
Some, but far from all dogs with a high Iodine intake in this developed lymphocytic thyroiditis.

FURTHER STUDIES

Further analyses to reveal possible interactions between Iodine intake and a genetic predisposition to develop canine lymphocytic thyroiditis will be performed when genetic risk haplotypes besides DLA class II have been identified by ongoing GW analyses and finemapping.

In order to further investigate the non-genetic risk factors for autoimmune thyroiditis in dogs, a prospective study would be necessary including analyses of drinking water, feed and additives from pregnancy and lactation of the bitch and several years ahead in each dog. Ideally this should be performed in combination also with other environmental effects that have been proven to have an effect in the development of autoimmune thyroiditis in man; i.e low birth weight, allergy, stress, passive smoking and previous infections.

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Hundägarenkät angående foder och tillskott

Hundens namn_____ reg. Tilltalsnamn_____

Reg nr_____ Lever hunden idag? □ Ja □ Nej

Ras: □ Riesenschnauzer □ Hovawart Kön: □ Tik □ Hane

1. **Hälsa**
   a. Är hunden diagnosticerad med sköldkörtelrubbning? □ Ja □ Nej
   b. Har hunden visat något av följande symtom: □ Håravfall □ Viktökning □ Slöhet
   c. Har hunden, eller har den tidigare haft några andra allvarliga eller kroniska sjukdomar? Om ja, vad?_____
   d. Går hunden eller har hunden gått på långvarig medicinering, vilken?_____
   e. Har hunden gått i avel? □ Ja □ Nej
2. **Foder**
   a. Vad åt hunden som valp (2-6 mån)? Kontrollera om det foder som utgjorde valpens huvudsakliga näringskälla finns med i tabellen på baksidan och markera utfodringen under den perioden med nr 1 i rutan. Om Din hund under perioden ätit flera olika foder Ange dessa som 1a,b, c, d…….
   b. Vad åt hunden som unghund (7-24 mån)? Markera med nr 2 i rutan i tabellen.
   c. Vad har hunden ätit som vuxen (> 2 år)? Markera med nr 3 i rutan/rutorna i tabellen.
   d. Ange hur länge hunden ätit detta foder?_____
   e. Om det foder hunden äter/ätit inte finns med i tabellen, ange både fabrikat och sort nedan följt av siffror och bokstäver enligt ovan:
      Fabrikat _____
      Sort (ex performance, lamb & rice)_____
3. **Tillskott**

   Får hunden eller har hunden fått tillskott? Markera med 1-4 i tabellen.

   1 = valp (2-6 mån) 2 = unghund (7-24 mån) 3 = vuxen (> 2 år) 4 = idag

□ Alg-Börje □ Annan algprodukt	□ Kafomavit	□ Dogevit □ Dogevit elit	□ Plaque-off
<u>Aptus</u> □ Multidog □ Multidog vita □ Multipuppy	□ Standardt Vilken sort: _____	<u>Versele-Laga</u> □ Orapharma Dog Form vitaminer	

Annat tillskott än de som står i tabellen ovan (t ex laxolja):_____

Hur stor mängd av tillskottet har hunden fått per dag (ex. antal mått, tabletter, krm) och under vilken period?_____

Stort tack för er medverkan!

Doggy ___ Orginal ___ Prima ___ Junior ___ Light ___ Energi ___ Senior	Doggy Proffessional ___ Extra Valp ___ Extra Skonsam ___ Extra ___ Extra Aktiv	Robur ___ Breeder & Puppy ___ Breeder & Puppy XL ___ Maintenance ___ Genuine Lamb & Rice ___ Genuine Salmon & Rice ___ Performance ___ Active & Sensitive ___ Light & Sensitive	Bozita ___ Junior ___ Active ___ Orginal ___ Orginal Mini ___ Orginal XL ___ Orginal Plus ___ Senior ___ Sensitive Lamb & Rice ___ Light Chicken & Rice
Vov ___ Färs med kött	___ Revelj	Revir ___ Bas ___ Sport	Frolic ___ Oxkött ___ Kyckling
Best in show ___ Valp Mini ___ Valp Maxi ___ Fullkost ___ Lamm & Ris ___ Lamm & Ris Light	Axcess ___ Premium ___ Kennel ___ Hundfoder ___ Light ___ Allround	Carrier ___ Orginal ___ Regular ___ Super Premium ___ Lamm & Ris ___ Kyckling & Ris ___ Light/ Senior ___ Junior	Magnusson Meat & Biscuit ___ Adult ___ Light ___ Work ___ Junior Orginal ___ Kennel ___ Lätta ___ Aktiv ___ Den naturliga hundmaten
Klass ___ Normal ___ Fisk ___ Plus	___ Friskies	___ Kompis	Royal Canin ___ Rasfoder
Royal Canin Mini ___ Beauty ___ Sensible ___ Dental Health ___ Light ___ Mature ___ Junior ___ Adult	Royal Canin Medium ___ Junior ___ Adult ___ Light ___ Mature ___ Sensible ___ Club Adult Croc	Royal Canin Maxi ___ Babydog ultra ___ Junior ___ Adult ___ Light ___ Mature ___ Sensible ___ Exclusive protein Adult ___ Exclusive protein Junior	Royal Canin Giant ___ Babydog ultra ___ Puppy ___ Junior ___ Adult Performance ___ Energy 4300 ___ Energy 4800
Eukanuba Puppy & Junior ___ Lamb & Rice ___ Large ___ Medium ___ Small	Eukanuba Wild nature ___ Salmon ___ Turkey ___ Venison	Eukanuba Everyday ___ Large ___ Small & Medium ___ Low activity	Eukanuba Sensitive ___ Skin ___ Digestion ___ Joints
Eukanuba Adult Lamb & Rice ___ Large ___ Small & Medium Adult Light ___ Large ___ Small & Medium	Eukanuba Adult Mature & Senior ___ Large ___ Small & Medium ___ Lamb & Rice	Eukanuba Adult ___ Large ___ Medium ___ Small	Eukanuba ___ Working & Endurance ___ Jogging & Agility ___ Excess Weight ___ Senior Plus ___ Rasfoder

Purina Pro Plan Adult ___ Lamb & Rice ___ Large Athletic ___ Large Robust ___ Original ___ Sensitive ___ Small ___ Small sensitive	Purina Pro Plan ___ Performance Light ___ Digestion ___ Original Senior ___ Sensitive ___ Original	Purina Pro Plan Puppy ___ Small ___ Sensitive ___ Original ___ Digestion ___ Large Athletic ___ Large Robust	Hill's Science Plan Mature Adult ___ 7+ Lamb & Rice ___ 5+ Large Breed ___ 7+ Mini ___ 7+ Light ___ 7+ Active ___ Performance
Hill's Science Plan Adult advanced fitness ___ Lamb & Rice ___ Large Breed ___ Large Breed – Lamb ___ Medium ___ Mini ___ Tuna & Rice	Hill's Science Plan Adult ___ Light ___ Light Large Breed ___ Oral Care ___ Sensitive Skin ___ Sensitive Stomach	Hill's Science Plan Puppy ___ Lamb & Rice ___ Large Breed ___ Medium ___ Mini	Hill's Science Plan Nature's Best adult ___ Large/ Giant ___ Mini/ Medium ___ 7+ Mini/ Medium Nature's Best puppy ___ Large/ Giant ___ Mini/ Medium
Nutra Nuggets ___ Lamb meal & Rice ___ Large Breed puppy ___ Large Breed adult ___ Puppy ___ Maintenance ___ Performance ___ Professional ___ Lite/ senior	Nutra Gold ___ Lax och potatis ___ Large Breed ___ Adult ___ Puppy ___ Senior ___ Lamb & Rice ___ Indoor microbites	Specific ___ Joint support ___ Weight reduction ___ Weight control ___ Active Large & giant breed ___ Puppy ___ Adult ___ Senior	Specific Medium breed ___ Puppy ___ Adult ___ Senior Small breed ___ Puppy ___ Adult ___ Senior
Pedigree ___ Junior ___ Adult ___ Senior ___ Large dog ___ Light ___ Active Better by nature ___ Junior ___ Adult	Nutro Choice Puppy ___ Mini ___ Lamb & Rice ___ Large Breed Adult ___ Large Breed ___ Lamb & Rice ___ Performance	James Wellbeloved Lamb & Rice ___ Puppy ___ Junior ___ Adult ___ Light/ Senior ___ Large kibble Lamb & vegetables ___ Adult	James Wellbeloved Turkey & Rice ___ Puppy ___ Junior ___ Adult ___ Light/ Senior ___ Large kibble
James Wellbeloved Duck & Rice ___ Puppy ___ Junior ___ Adult ___ Light/ Senior	ANF Holistic ___ Puppy ___ Adult ___ Duck & Potatoes ___ Fish meal & Potatoes	ANF ___ Puppy ___ Puppy 28 ___ Adult Chicken & Rice ___ Adult Turkey & Barley ___ Adult Lamb & Rice ___ Canine Low Activity ___ Performance	Lone Star ___ Puppy ___ Healthy Choice ___ Adult ___ Lamb & Rice ___ Magnum ___ Performance ___ Senior
Precept ___ Plus Puppy ___ Plus Adult ___ Puppy ___ Growth ___ Foundation	Precept ___ Sensicare ___ Chicken & Rice ___ Competition ___ Endurance ___ Light	Advance Maxi ___ Puppy ___ Junior ___ Adult ___ Light	Advance ___ Maxi senior ___ Performance