Epidemiological Aspects of Feline Hyperthyroidism in New Zealand

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Abstract

A questionnaire-based case-control study of 375 cats was conducted in New Zealand over a 14-month period from 1996 to 1998 and then used to identify possible risk factors for feline hyperthyroidism.

The owners of 125 hyperthyroid cats, 125 age- and sex-matched and 125 random control cats were asked 64 questions, about their cats' exposure to potential risk factors including: cat and owner demographics, each cat's medical history, the indoor and outdoor environment, the cat's diet and feeding practices.

For the clinical cases a questionnaire of 10 questions completed by the attending veterinarian provided the demographic data, the cat's medical history and clinical signs before, and at the time of diagnosis of hyperthyroidism.

A range of statistical techniques was employed to analyse the data, including univariate odds ratio and chi-squared calculations, stepwise forward unconditional (case-random controls) and conditional (case-matched controls) logistic regression, frequency analyses and Cox regression (proportional hazards model) for case-random status. Kaplan-Meier survival analysis was used for hyperthyroid cats to evaluate the effects of a number of different treatments, including medical, surgical and radioactive iodine treatment, on survival time (months) of the cats after the diagnosis and up to the final date of the study.

Variables that were positively associated with feline hyperthyroidism from the case-random control comparison included age, breed, sex, age at desexing, history of any oral cavity diseases, sleeping predominantly on the floor, regular use of anti-flea products (in particular applied to the cats'

bed/bedding) and eating more than ½ of the daily diet as a commercial canned food.

Older cats were more likely to develop hyperthyroidism. Siamese cats were found to have a lower risk for developing hyperthyroidism. Females were three times as likely to develop the condition as males. With cat's age at desexing, the category "don't know", which indicated either that the cat had had a previous owner or was of unknown origin, was associated with increased risk for developing hyperthyroidism. Although oral cavity diseases were controlled for age, the occurrence of dental disorders was associated with a five-and- a-half-fold higher risk of developing hyperthyroidism. A 6.6-fold increase in risk of developing hyperthyroidism was calculated for cats sleeping predominantly on the floor. Cats eating half or more of their daily food as a canned commercial cat food were shown to have twice the risk of developing hyperthyroidism as those cats whose diets excluded canned food.

In order to focus on factors which might influence occurrence of the disease in cats at similar constitutional risk of feline hyperthyroidism a second investigation was conducted in which each case was compared with a control animal matched on sex and age (± 1.5 years) for the case.

In this comparison, cats with episodes of diarrhoea were seven times more likely to have hyperthyroidism. The use of fly sprays in the cat's indoor territory was also associated with an increased risk of developing this disorder. Cats eating a variety of flavours of commercial canned cat food had 3.8-fold increased risk of developing hyperthyroidism compared with cats whose diets consisted of a single flavour of canned food. The interaction between drinking water from puddles and the regular use of animal/plant origin fertilisers (sheep manure, compost, commercial blood

and bone fertiliser) in the cat's outdoor territory was associated with a 5.3-fold higher risk of developing disease.

Other variables that appeared to have some protective effects included "more than one cat in the household" (from the case-matched model) and the previously mentioned protective effect of breed, for Siamese cats only, from the case-random control comparison.

The questionnaire completed by veterinarians provided information on history and clinical findings in affected cats. The frequencies for the clinical signs weight loss, polyphagia, hyperactivity, tachycardia and palpable thyroid gland(s) were 92%, 68%, 34%, 62% and 56% respectively. Skin changes, episodes of vomiting and decreased activity had the following frequencies: 49%, 26% and 11% respectively.
The increased number of feline hyperthyroidism diagnoses in the warmer six months of the year, from October until March, indicates seasonality of recognition of disease, but may not represent true date of onset.

The relevance of the identified risk factors to the aetiology of feline hyperthyroidism is discussed, bearing in mind that some of the potential risk variables mentioned earlier could be the result of the disease itself. The analysis of this study suggested that further investigations should be undertaken into the molecular basis of the disease, into dietary factors and other potentially important risk factors such as insecticides, breed and sex susceptibility.

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Dedication

This thesis is dedicated to all the cats of the world, small and big, domestic and wild, in particular to the late Mimusia, Spust and Simon

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Chapter 1

Introduction

General introduction

Feline hyperthyroidism (thyrotoxicosis) is a multisystemic disease resulting from excessive secretion of thyroid hormones, L-thyroxine (T_4) and/or L-triiodothyronine (T_3). This disorder is now the most common endocrine disease of middle-aged to older domestic cats (average age at diagnosis of 12 to 14 years) diagnosed at veterinary clinics in New Zealand and many other countries (Labuc and Jones, 1988; Peterson, 1984). The most common clinical signs are weight loss, polyphagia and hyperexcitability.

A number of suspected cases were reported in the veterinary literature from 1955 onwards but the first confirmed cases were those of Holzworth *et al.* (1980), of Cotter, and of Peterson *et al.* in 1979. Despite progress in improving the diagnosis and treatment of feline hyperthyroidism, it has been observed that over time there has been an increase in the incidence of this disease (Scarlett *et al.*, 1988; Taylor *et al.*, 1989). The disease frequency suggested by Peterson (1984) is one in 300 cats examined at the Animal Medical Centre (AMC), New York (U.S.A.). AMC used to diagnose 3 cases per month (1978-1983); later 22 cases per month were diagnosed (1983-1993) without a concomitant increase in total case load or mean age of cats seen in consultations (Peterson, 1998; Peterson *et al.*, 1994). The frequency of occurrence of feline hyperthyroidism in New Zealand is not known.

The factor(s) initiating and sustaining the transformation of a normal feline thyroid into a nodular hyperfunctioning goitre remain unknown. Genetic predisposition, environmental exposure to some chemical(s), immunological factors, nutritional constituents and perhaps infectious agent(s) may all influence the development of the disease (Gerber, 1993;

Gerber *et al.*, 1994; Jones *et al.*, 1995; Luttikhuis, 1989; Scarlett, 1994; Scarlett *et al.*, 1988; Taylor *et al.*, 1989; Thoday and Mooney, 1992).

Scarlett and co-workers (1988) identified an increased risk for hyperthyroidism associated with regular treatment with flea sprays or powders; living strictly indoors; and having reported exposure to lawn herbicides, fertilisers or pesticides. Cats whose diets were 1/2 or more canned food had 3.4 times as high a risk of developing this disorder compared with those fed no canned food; but cats fed less than ½ canned food had 1.6 times the risk of those fed no canned food at all.

There is no reported sex or breed predisposition, but a lower prevalence in purebred cats has been noticed in the U.S.A. (Scarlett *et al.*, 1988) and UK (Blaxter and Gruffydd-Jones, 1994). The high percentage of castrated and ovariohysterectomised animals in the samples so far examined makes conclusions about sex differences weak in inferential value.

The reported age range has widened since the first reports of the disease. It now spans from 4 to 22 years (Peterson *et al.*, 1994), with an average age of 12 to 14 years.

There are some data to suggest that commercial diets may play a role. There is abundant evidence *in vivo* and *in vitro* for a role of iodine as a regulator of thyroid growth and function (Studer and Gerber, 1991).

The concentration of iodine varies widely in commercial cat food in the U.S.A. (Mumma *et al.*, 1986) and in New Zealand (Johnson *et al.*, 1992), with some foods containing very small amounts and others far above current recommendations for cats (up to 10 times) (AAFCO, 1993; Johnson

et al., 1992). Johnson and co-workers' (1992) study of commercial cat food preparations showed that the iodine concentration can vary more than 100-fold.

In canned pet foods, there has been a trend towards a lower ash content (mainly magnesium) and towards gourmet products containing more animal tissue. Iodine concentrations may be higher in gourmet foods containing marine fish or poultry products with remains of thyroid tissue (Scarlett *et al.*, 1988).

As well as iodine, a xanthene dye, FD & C Red No. 3 (common name erythrosine), is added frequently to pet food. This artificial dye contains iodinated compounds, which release free iodine during processing, resulting in a higher concentration of this element in the food (Barbano and Dellavalle, 1984).

There is evidence that selenium plays an important role in thyroid hormone metabolism as an essential component of the three deiodinases (Arthur *et al.*, 1996). New Zealand's volcanic soils are, in general, deficient in selenium. The role of selenium is further complicated because of the widespread practice of adding synthetic antioxidants to pet food (Dodds, 1995). Synthetic antioxidants have the potential to change the bioavailability of vitamin A (essential for many biochemical pathways including thyroid metabolism), vitamin E and selenium.

Apart from iodine, most cat foods contain relatively high concentrations of goitrogenic compounds such as phthalates, which can be found in fish products, milk and bovine tissue (Mayer *et al.*, 1972). There are many other goitrogenic substances (Gaitan, 1988; Roudebush, 1993), which cats

may be exposed to, either through their diets (particularly fish-based meals) or in the environment, that could contribute to the development of the thyroid adenomatous lesions found in most hyperthyroid cats. Most of these goitrogenic hydrocarbons are metabolised by glucuronidation, a process which is exceptionally slow in cats.

In addition, naturally occurring goitrogens such as soy and some other beans, onion and garlic, seaweed (e.g. kelp), gums made from seaweed (carrageenan, alginates), and linseed are extensively used as ingredients in pet foods.

Some other dietary ingredients are goitrogenic too, e.g. calcium (at least in rats on a low-iodine diet), sulphur-containing compounds and nitrate found in drinking water (McLaren and Alexander, 1979).

Although it seems unlikely that dietary goitrogens acting alone can cause goitre, it is quite possible that their effects may be additive when interacting with other factors, such as iodine deficiency or excess, including day-to-day wide swings of iodine content in the diets. Other types of goitrogen from the environment (from water, air and food) may contribute to the overall increasing incidence of human goitre in endemic areas (Gaitan, 1988; McLaren and Alexander, 1979).

There is epidemiological and experimental evidence that environmental pollutants and certain medications may cause goitre by acting directly on the thyroid gland or by indirectly altering its regulatory mechanism and/or the peripheral metabolism and excretion of thyroid hormones. These pollutants operating in genetically predisposed individuals may also trigger

pathogenetic mechanisms in the thyroid gland much more easily than in less genetically susceptible ones (Gaitan, 1988).

All of the above hypotheses need to be clarified as to their potential association with hyperthyroidism in cats.

There are still many unanswered questions relating to the pathogenesis and epidemiology of feline hyperthyroidism. The lifestyle and feeding practices of cats in New Zealand have similarities to cats in other countries, but also differ in many important respects especially the case of access to outdoor activity. New Zealand has a high proportion of cat-owning households and the movement of cats into and out of country is not significant. Since late 1990/early 1991 the cat population in New Zealand has remained flat at around 0.9 million cats (EFS, 1997). The average number of cats per cat owning household has also stayed flat at around 1.55 cats per cat owning household. Though the number of households in New Zealand has been gradually increasing since 1990 at the rate of 1.3%, the number of households owning a cat remained the same (EFS, 1997). As a result the percentage of households owning a cat has dropped from 50% to 47%. However, the last figure is a lot higher than the percentage of cat ownership in Australia, which is about 30%. Most cats are indoor/outdoor cats and prior to 1990 commercial foods did not constitute a high proportion of any cat's diet. Cats in New Zealand provide a unique population to investigate the epidemiology of hyperthyroidism.

This study was designed to investigate and define some of the risk factors for feline hyperthyroidism in New Zealand.

Introduction to epidemiological studies

Epidemiological studies may not only reveal the frequency of a disease, but also give clues to possible associations with factors important for its occurrence. Epidemiological data could provide indications of what factors are associated with, or predispose to, the development of a particular disease. Also, epidemiological studies may answer important questions such as:

Is the occurrence of the disease in the population associated with the factors being identified?

Are certain combinations of factors especially significant, and how prevalent are they in the population?

Do the detected associations suggest a pathogenesis for the disease which, in turn, may lead to preventive measure?

In multifactorial diseases such as feline hyperthyroidism (Gerber *et al.*, 1994; Scarlett *et al.*, 1988; Taylor *et al.*, 1989), epidemiological studies become essential for investigations of the disease. This approach may provide a balanced view and analysis of the naturally occurring disease and its determinants may be identified. Husbandry and ecological factors, as well as the interactions between them are recognised and their importance identified.

Epidemiology is concerned with disease as it occurs in populations rather than in individual animals. However, the concept of population analysis has been rarely applied in small animal practice. Nevertheless, it is essential when veterinarians becomes active in preventive medicine, i.e. in

promoting general health and welfare in a specific area of animal population.

The two main objectives of epidemiological studies, namely, to describe the occurrence of disease in the population and to identify possible causal factors, may be reached by various designs, which can accomplish one or more objectives.

Cohort (incidence) studies are concerned with monitoring a defined population sample (two or more cohorts which differ in one or more putative risk factors) and comparing the incidence of disease between cohorts. After a period of observation, incidence rates are calculated and attributed to the presence or absence of the putative factors.

Cross sectional (prevalence) studies are concerned with examination of the relationship between the disease and the various potential risk factors as they exist in a defined population at one particular time. This is done by examining a sample of the population for the presence or absence of the disease and of suspected determinants.

Case control (case history) studies are concerned with comparing the distribution of individuals among factor categories in a diseased group (the case group) with the corresponding distribution in a group of non-diseased individuals (the control group or reference population).

With cohort studies results are given as incidence rates, while in cross sectional studies the results are given as prevalences. This approach does not apply to a case control study, as neither the case group nor the control group can be quantitatively related to the population at risk.

As meaningful disease rates cannot normally be estimated from case control studies, associations must be shown by means other than comparison of factor-specific rates, e.g. by relative risk estimations. Relative risk is a measure of disease association defined as the ratio of the disease rates for two categories of a factor, and it thus measures the disease association in one category using that of another category as a reference unit. Relative risk can be used to express factor associations in all three types of studies mentioned above, since it can be approximated from case control studies by the ratio of odds of the disease for the two categories, provided that the disease is uncommon in the population.

Chapter 2

Literature Review

Introduction

Feline hyperthyroidism (thyrotoxicosis) is a multisystemic disease resulting from excessive secretion of thyroid hormones, L-thyroxine (T_4) and/or L-triiodothyronine (T_3). This disorder appears to be the single most common endocrine disease of middle-aged to older domestic cats (average age of 12 to 14 years) diagnosed at the Massey University Small Animal Clinic as well as at a number veterinary clinics in several other countries.

A number of suspected cases were reported in the veterinary literature from 1955. Clark and Meier (1958) showed thyroid adenomas in 14.7% of feline post-mortem examinations, while Lucke (1964) in 30.6% of cat necropsies. The authors of these studies mentioned that the majority of cats with adenomas had no recognised clinical signs before death and that most of the thyroids were not grossly enlarged. However, some misdiagnoses of feline hyperthyroidism occurred, as careful review of the early pathological examinations reveals that some of the cats had clinical signs compatible with hyperthyroidism (Lucke, 1964). The first confirmed cases were those of Cotter (1979), Holzworth (1980), Jones and Johnstone (1981) and Peterson *et al.* (1979). Even considering progress in improving the diagnosis and treatment of feline hyperthyroidism, it has been observed that over time there has been a marked increase in the incidence of this disease (Scarlett *et al.*, 1988; Taylor *et al.*, 1989). The disease frequency suggested by Peterson (1984) is one in 300 cats examined at the Animal Medical Centre (AMC) in New York (U.S.A.). AMC used to diagnose 3 cases per month (1978-1983); later 22 cases per month were diagnosed (1983-1993) (Peterson *et al.*, 1994). Peterson (1998) pointed out that the increased number of hyperthyroid cats occurred without an accompanying increase in total case load or mean age of

cats seen in consultations at AMC. The frequency of occurrence of feline hyperthyroidism in New Zealand is not known.

The purpose of this chapter is to review the literature available on this topic, emphasising the aetiopathological and the epidemiological aspects of feline hyperthyroidism. There are very comprehensive and thorough literature reviews on feline hyperthyroidism written by Labuc and Jones (1986 and 1988), Peterson *et al.* (1994) and Thoday (1988).

Aetiology, pathology and molecular aspects

Hyperthyroid cats, in addition to their clinical relevance for veterinary medicine, provide a unique experimental model to investigate the mechanisms underlying thyroid hyperplasia and hyperfunction because of the close resemblance to human toxic nodular goitre.

The factor(s) initiating and sustaining the transformation of a normal feline thyroid into a nodular hyperfunctioning goitre remain unknown. Genetic predisposition, environmental exposure to some chemical(s), immunological factors, nutritional constituents and perhaps infectious agent(s) may all influence the development of the disease (Gerber, 1993; Gerber *et al.*, 1994; Scarlett *et al.*, 1988; Taylor *et al.*, 1989).

Functional thyroid adenoma (multinodular adenomatous hyperplasia) involving one (21% to 29%) or both thyroid lobes (71% to 79%) is the most common cause of feline hyperthyroidism (97% to 99%) (Hoenig *et al.*, 1982; Holzworth *et al.*, 1980; Peterson *et al.*, 1983). In unilateral multinodular adenomatous goitre left lobe is involved in 58%, while right lobe in 42%. Thyroid adenocarcinoma, the primary cause of

hyperthyroidism in the dog (very rare in fact), rarely causes hyperthyroidism in cats (1% to 3%) and when present seldom metastasises (Hoenig *et al.*, 1982).

Thyroid microsomal (anti-thyroid peroxidase, anti-TPO) and anti-nuclear (tissue-nonspecific, ANA, rare in healthy cats) antibodies have been demonstrated in sera from 29 hyperthyroid cats, in 34% and 14% respectively (Kennedy and Thoday, 1988). The correlation between lymphocytic infiltration (i.e. autoimmune thyroiditis) of the gland, in most cases with bilateral involvement, and circulating thyroid autoantibodies is significant ($p < 0.05$, Kennedy and Thoday, 1988). This phenomenon is well recognised in human Graves' hyperthyroidism and the presence of antibodies is used as a prognostic tool (McKenzie and Zakarija, 1991). In addition, Kennedy and Thoday (1984) showed that 48% of 29 studied cats had circulating anti-thyroglobulin antibodies.

Studies by Luo *et al.* (1993 and 1994) showed that immunisation of mice with the enterobacterium *Yersinia enterocolitica* leads to the production of antibodies against the human TSH receptor (TSHR). The results suggested that molecular mimicry, between *Y. enterocolitica* envelope proteins and the TSHR, might play a role in the induction of autoantibodies to TSHR as occurs in Graves' disease. There has not been any research on immunological cross-reactions between any infectious agent(s) and feline hyperthyroidism.

Anti-TSH (thyrotropin - thyroid stimulating hormone) receptor antibodies or TSAb (thyroid stimulating antibodies) or TSIs (thyroid stimulating immunoglobulins), as found in human patients with Graves' disease, were not found in sera from affected cats (Peterson *et al.*, 1987; Thoday, 1988)

but thyroid growth stimulating immunoglobulins (TGIs) were identified (Brown *et al.*, 1992). These IgG immunoglobulins, presumably working through the TSH receptor, act to promote thyroid growth but do not stimulate thyroid hormone secretion. The pathogenic relevance, if any, of thyroid autoantibodies has not been established in feline hyperthyroidism.

Additionally, the results of a recent investigation conducted in the United States suggest that the hyperthyroid cats did not develop stimulatory autoantibodies against the thyroid stimulating hormone (TSH) receptor (Nguyen *et al.*, 1998). In this study Nguyen did molecular cloning of a full-length cDNA of the feline TSH receptor (TSHR) by reverse transcriptase – polymerase chain reaction (RT-PCR). This cDNA sequence contained an open reading frame of 2292 nucleotides and encodes a 763 amino acid protein, one amino acid less than the human TSH receptor. In comparison to the human TSH receptor, the amino acid sequence was of 90% identity and 92% similarity. When the species comparison was performed, it revealed that the cat TSH receptor was most closely related to the canine TSH receptor sequence (96% identity, 97% similarity). The functional expression of the cat TSH receptor by transfection in a heterologous system using a cAMP-dependent luciferase reporter assay revealed similar responsiveness to exogenously administered bovine TSH in comparison to the human TSH receptor. Serum collected from six hyperthyroid cats did not result in increased cAMP accumulation in cells transfected with the feline TSH receptor and consequently it can be deducted that these hyperthyroid cats did not develop stimulatory autoantibodies against the TSH receptor (TSHR). Binding of I^{125}-labeled bovine TSH was similar for the feline and the human receptor. This observation provided further evidence against circulating thyroid

stimulating factors as a mechanism underlying feline hyperthyroidism and against an autoimmune aetiology of that condition.

When Peter *et al.* (1986) transplanted thyroid tissue from hyperthyroid cats into athymic nude mice, the transplants grew and continued to overproduce thyroid hormone in exactly the same way as in the donor tissue, and as in a similar study on human patients with toxic nodular goitre. Feline toxic nodular goitre tissue xenotransplanted into thyroxine-treated nude mice retained its original histological pattern and continued to accumulate I^{125} intensely and autonomously, i.e. in the absence of TSH (Faber and Rubin, 1991). Furthermore, administration of sera from hyperthyroid cats into host mice failed to stimulate the xenografts.

Those findings indicate that both growth and hyperfunction of cat goitres are intrinsic properties of the thyroid tissue and are not the result of an external immunoglobulin stimulator - a circulating extrathyroidal stimulator (Faber and Rubin, 1991; Nguyen *et al.*, 1998; Peterson *et al.*, 1983). Humoral factors may be involved in the initiation but not in the maintenance of the condition (Peter *et al.*, 1986). A very weak thyroid growth stimulator (e.g. thyroid growth stimulating immunoglobulins [TGIs]) enhancing the transformation of the normal gland into a nodular goitre over many years could play an initiating role. Another possibility is that thyroid cells might produce growth factors resulting in continued proliferative responses in vitro (Gerber *et al.*, 1994). The basic lesion appears to be an excessive intrinsic growth capacity of some thyroid cells. Feline hyperthyroidism resembles human toxic nodular goitre (Plummer's disease), in which hormone hypersecretion is caused by the TSH-independent overactivity of a great number of so-called autonomous follicles (Studer *et al.*, 1989).

Toxic nodular goitre is characterised by two types of "thyroid autonomies". "Thyroid autonomy of growth" (i.e. TSH-independent; "autonomy from TSH") is represented by subpopulations of follicular cells with a massive growth potential. Second, "autonomy of function" is represented by higher number of autonomously functioning follicles with a much higher degree of autonomous iodine turnover, i.e. within the "hot" goitre area (Studer and Gerber, 1991).

These intrinsic properties of individual thyroid cells are explained by genetically determined cellular heterogeneity. Heterogeneity applies to the thyrocytes' growth potential and their functions such as iodination, peroxidase content, thyroglobulin (Tgb) synthesis and endocytosis (Studer and Gerber, 1991).

The nodular growth pattern of the thyroid gland in humans can be illustrated by three mechanisms explained below (Studer and Gerber, 1991). This model is typical for long-standing goitres and for normal thyroids in old, healthy people.

Firstly, some thyrocyte subpopulations are characterised by growth advantage and they have a tendency to remain clustered causing focal hyperplasia, and finally appearing as nodules. In human goitres this focal hyperplasia has been linked to the expression of the *ras* protooncogene product *p21* (Huber *et al.*, 1990).

Secondly, the nodule formation could be the result of a somatic mutation presenting a heritable growth advantage to a single cell. The two mechanisms could be linked sometimes. Knowing the increased predisposition of fast-growing cells to obtain any kind of genetic defect, for example of growth regulation, Studer and Gerber (1991) suggested that adenomas and even malignant thyroid tumours may be due to presence of

cell subpopulations with a high intrinsic proliferation rate. This concept was supported by the research of Faber and Rubin (1991) and of Groch and Clifton (1992).

Thirdly, the mechanism of nodular growth is caused by the network of fibrous strands which result from scarring, necrosis and haemorrhages which occur in most growing goitres.

The above described observations support a model of intrinsic autonomy of thyroid follicular cell function as a mechanism underlying feline hyperthyroidism. This model still remains to be defined at the molecular level.

The first insights into thyrotropin stimulating receptor (TSHR) molecular structure have been carried out already. For instance, in humans activating TSH receptor germline mutations account for the majority of autonomous adenomas, and John Morris at Mayo (Grebe, 1998) has found a high frequency of a certain germline polymorphism in human multinodular goitres. However, the functional significance of the germline polymorphism in humans is not known yet. Assuming that cats might be similar, the next step would be to sequence the feline TSH receptor and screen for mutations.

Pearce *et al.* (1997) already used polymerase chain reaction (PCR) to amplify codons 480–640 of the feline thyrotropin receptor (TSHR) gene and have determined the sequence in the transmembrane domain region. The normal feline TSHR sequence between codons 480–640 is highly homologous to that of other mammalian TSHRs, with 95%, 92% and 90% amino acids identity between the feline receptor and the canine, human and bovine TSHRs respectively. Please note that the figures just mentioned are very close to figures of Nguyen *et al.* (1998). Thyroid gland DNA from 11 cats with sporadic hyperthyroidism did not have mutations in this region

(transmembrane domain) of the TSHR gene. Additionally, leukocyte DNA from two cases of familial feline thyrotoxicosis did not harbour mutations of this region of the TSHR gene. Therefore, Pearce *et al.*, (1997) study suggested that TSHR gene mutations are not a common cause of feline hyperthyroidism and as mentioned earlier the functional significance of the germline polymorphism in humans is not known, either. Therefore further investigations are warranted.

There has not been any work done to evaluate thyroid histology and function in older cats without clinical signs, except for one study by Lucke (1964).

All of the changes mentioned earlier within multinodular adenomatous goitre will lead in the end to excessive hormone production by autonomous follicles and hence a simple goitre becomes toxic nodular goitre. At this point, endogenous TSH secretion is shut off and hormone production in normal follicles is reduced to its lowest possible level (always something above zero) (Studer *et al.*, 1985). This process can take many months or years before being expressed as disease.

In addition, Luttikhuis (1989) and Thoday (1992) reported hyperthyroidism in a queen and her two male offspring, and in two female siblings. This familial prevalence could indicate the existence of an inherited predisposition to this disorder. Since these cats were raised in the same households, they had common exposure to environmental factor(s)/toxin(s). Thus the possibility of the horizontal transmission of some unidentified infectious agent(s) or other environmental factors could not be excluded.

Finally, Jones and colleagues' (1995) study on prevalence of feline immunodeficiency virus infection in hyperthyroid cats did not support involvement of that virus in the pathogenesis of feline hyperthyroidism.

History and clinical findings

Two outstanding characteristics of thyrotoxicosis in nodular goitre are the insidious clinical onset, probably spanning many months, years or (in man) even decades; and its progressive course. Clinical signs vary from mild to severe, depending on the duration of the condition, the ability of the cat's body to cope with the demands imposed by thyroid hormone oversecretion, and the presence of a variety of other diseases, which are common in older cats (Peterson *et al.*, 1983).

The clinical presentation is usually multisystemic and the differential diagnoses for hyperthyroidism are quite extensive (Labuc and Jones, 1986 and 1988).

A hyperthyroid cat with a typical history and physical examination findings (including a palpable thyroid gland, rapid heart rate (> 200 beats per minute), emaciation and a high serum thyroid hormone concentration) represents a highly recognisable, familiar syndrome (Broussard and Peterson, 1993; Broussard *et al.*, 1995; Hoenig *et al.*, 1982; Holzworth *et al.*, 1980; Labuc and Jones, 1986 and 1988; Peterson *et al.*, 1983 and 1994; Thoday, 1988; Thoday and Mooney, 1992). Table 8, presented in the discussion, shows that the frequency of many historical and clinical signs in the 1993 report was considerably decreased compared with the early 1980s. The typical hyperthyroid cat of today tends to show less severe clinical signs than one diagnosed a few years ago. The severity of feline

hyperthyroidism is thought to have reduced over the last 12 to 18 years since the first cases were reported, but clinical signs and physical examination findings still remain useful indicators of this disorder. The most likely explanation for the reduction in severity of clinical signs is higher awareness among veterinarians of that disease and in general better diagnosis skills.

The diagnosis can be more difficult when a cat is presented in the early stages of the disease (Turrel, 1992) or when the apathetic (masked) form, which occurs in 10% of affected animals, is seen (Peterson *et al.*, 1983), or when severe concurrent nonthyroidal illness is present (Ferguson, 1996; Peterson and Gamble, 1990; Turrel, 1992). Peterson and Gamble (1990), and Mooney *et al.* (1996) found that nonthyroidal diseases can seriously decrease circulating thyroid hormone concentrations but certain ailments are more consistently associated with this finding, for example diabetes mellitus, renal failure, liver disease and systemic neoplasia. The severity of nonthyroidal disorders has a more significant ($p < 0.001$) effect in lowering serum total T_4 concentrations than does the disease category. Also, a low total T_4 concentration in severe medical illness in cat and man predicts higher mortality rates. Despite low serum total T_4 concentrations, euthyroidism is maintained in sick cats because serum free T4 concentrations tend to remain within the reference range (Mooney *et al.*, 1996).

Additionally, in some cats with early or mild hyperthyroidism, concomitant nonthyroidal disease may suppress serum T_4 concentrations into the reference range. These false negative results may be influenced by thyroid hormone fluctuations or previous drug therapy as well. The day-to-day fluctuations were found to be greater than within-day variations (Peterson *et al.*, 1987). The following drugs have the potential to depress thyroid

hormone concentrations: glucocorticoids, sulphonamides, iodine-containing agents, radiocontrast agents, dopamine, beta blockers (mainly propanolol). General anaesthesia, mainly when anaesthetic agents containing a thiocarbamate structure (e.g. thiopental) are used, can lower serum T_4 and T_3 for as long as a week (Wase and Foster, 1956). On the other hand, some drugs are able to increase thyroid hormone concentrations, such as phenobarbital, rifampin, oestrogens and heparin (Ferguson, 1996). Moreover, false positive results may be due to presence of T_4 autoantibodies.

A large number of diagnostic tests have been applied to the investigation of feline hyperthyroidism (Eckersall *et al.*, 1991; Labuc and Jones, 1986 and 1988; Mooney *et al.*, 1996a; Nichols, 1996; Sparkes *et al.*, 1991; Taylor *et al.*, 1989; Thoday, 1988; Turrel, 1992). The laboratory data supporting a diagnosis based on history and clinical signs should allow treatment of only correctly identified hyperthyroid cats. Three options are available for treatment of this disorder: radioactive iodine (I^{131}), surgical thyroidectomy or administration of antithyroid drugs. The treatment and factors influencing the selection of appropriate method for each case, side effects and complications are well presented in papers written by Blaxter and Gruffydd-Jones (1994), Ferguson (1986), Jones *et al.* (1991), Labuc and Jones (1986 and 1988), Peterson *et al.* (1994), Thoday (1988) and Turrel *et al.* (1984).

Epidemiology of thyrotoxicosis due to nodular goitre in men and cats

The incidence of thyrotoxicosis caused by nodular goitre in human adults varies highly from one country to another, e.g. in U.K. the prevalence is

estimated from 5% to 8%; in Zürich and Hamburg from 30 to 40% (Studer *et al.*, 1985). While iodine deficiency may have favoured the incidence of nodular goitres in Zürich, Hamburg has never been considered as an endemic goitre area. Although, it was thought that toxic nodular goitre did not occur in young adults, in fact it does and has been diagnosed in humans aged 10 to 15 years (Studer *et al.*, 1985). As with most other thyroid diseases in people, the incidence of toxic nodular goitre is three to five times higher in females than in males.

There have been three recent investigations of potential risk factors associated with feline hyperthyroidism. Both descriptive and analytic studies have provided insight into the epidemiology of the disease. The age range became wider since the first reports of the disease for affected cats and it now spans from 4 to 22 years, with an average age of 12 to 14 years.

In a study of 56 cats with hyperthyroidism and 117 matched controls, Scarlett and co-workers (1988) identified an increased risk for hyperthyroidism associated with regular treatment with flea sprays or powders; living strictly indoors; having reported exposure to lawn herbicides, fertilisers or pesticides. Cats fed a majority of canned food in their diet had a 3.4 times greater risk of developing this disorder. The aetiological significance, if any, of these associations was not determined.

There is no sex or breed predisposition but a lower prevalence in pure breed cats has been noticed in the U.S.A. (Scarlett *et al.*, 1988) and U.K. (Blaxter and Gruffydd-Jones, 1994). For example, Scarlett and co-workers (1988) found that Siamese cats have a 10-fold lower likelihood of developing the disease. It may provide evidence for genetic factor(s) associated with this condition, or indicate the lack of exposure to the

aetiologic agent(s), or just reflect differences in the life expectancy of pedigree compared with mixed breed cats. The high percentage of castrated or ovariohysterectomised animals taking part in all so far completed studies, may show observations on the sex ratio to be invalid.

That lower prevalence in pure-breed cats has been noticed also in a second epidemiological study completed by Kass and co-workers in 1998. In a study of 379 cats with hyperthyroidism and 351 random controls Kass *et al.* (1998) noticed that two genetically related cat breeds, i.e. Siamese and Himalayan, had a diminished risk of developing hyperthyroidism. Cats which used litter had a 3-fold increased risk of developing hyperthyroidism compared with those which did not. Like Scarlett, Kass *et al.* (1998) found that cats which ate commercially prepared canned food had an approximately 2- to 3-fold increase in risk of disease compared with cats whose diets excluded canned food. In contrast to Scarlett, the use of commercial flea products was not significant.

The third epidemiological study was the one reported here, conducted in New Zealand in 1997. By and large, the findings confirmed some of the previous studies and suggested new avenues for further research.

The published incidence of feline hyperthyroidism from U.S.A. was highly variable around 1979 (Hoenig *et al.*, 1982; Holzworth *et al.*, 1980; Peterson *et al.*, 1983; Scarlett *et al.*, 1988). Most United States veterinary schools were reporting this condition by 1980. The number of cats over 7 years of age presented for veterinary care increased slowly since 1978 (Scarlett *et al.*, 1988). Later, reports of this disorder came from Europe (Thoday, 1992) and Australasia (Johnson *et al.*, 1992; Jones and Johnstone, 1981; Jones *et al.*, 1995; Tarttelin *et al.*, 1992). If some environmental exposure

or change in diet precipitated the onset of this disease, it is likely that those two facts occurred in a relatively short time and spread world-wide. Another possibility is that cats are living longer and owners are presenting them more frequently to veterinary clinics.

There are some data to suggest that commercial diet may play a role. There is abundant evidence *in vivo* and *in vitro* for a role of iodine as a regulator of thyroid growth and function (Studer and Gerber, 1991).

Iodine itself is able to modify the effects of growth factors on the thyroid gland (Studer and Gerber, 1991). The presence of iodine can decrease the number of thyroidal receptors for epithelial growth factor (EGF) (Miyamoto *et al.*, 1988) or even inhibit the growth of feline thyroid cells lines (Gerber *et al.*, 1991). Transforming growth factor-β, a well-known inhibitor of cell proliferation, is decreased in multinodular goitres because iodine induces its production (Bidey, 1990).

The concentration of iodine varies widely in commercial cat food in U.S.A. (Mumma *et al.*, 1986) and New Zealand (Johnson *et al.*, 1992), with some foods containing very small amounts and others far above current recommendations for cats (up to 10 times) (AAFCO, 1993; Johnson *et al.*, 1992). Johnson and co-workers' (1992) study of commercial cat food preparations showed that iodine concentration can vary more than 100-fold.

Excessive iodine ingestion can have a variety of physiological effects. The outcome depends on the functional state of the thyroid at the time of ingestion. Administration of iodine can induce hyperthyroidism in human patients with non-toxic nodular goitre living in areas with sufficient dietary iodine (Roti and Vagenakis, 1991), in healthy people living in endemic

iodine-deficient areas (Roti and Vagenakis, 1991) and in euthyroid patients with autonomously functioning thyroid follicles (Blum *et al.*, 1976).

Lower fractional rates of iodide loss occur in dogs and presumably in cats than in humans. Such losses are reflected by lower canine and feline clearance rates and longer total mean residence times, and thus the high serum iodide levels and iodine intake in cats may contribute to feline hyperthyroidism (Kaptein *et al.*, 1994). Tarttelin *et al.* (1992) revealed that the cat's thyroid hormone homeostasis is very sensitive to iodine intake, most likely explained by "Wolff-Chaikoff effect", a mechanism by which inorganic iodide uptake results in transient inhibition of organic iodide and reduced hormone synthesis. The short-term (2 weeks) feeding of canned cat food of widely differing iodine content resulted in a dramatic thyroid response as measured by serum free T_4 (i.e. the higher iodine intake, the higher the urinary iodine excretion, the lower the T_4 concentration). That thyroid response (decreased T_4 concentration) may result in permanent thyroid disease, such as nodular goitre with or without hyperthyroidism. Another study (Kyle *et al.*, 1994) showed that long-term (5 months) feeding of canned cat food varying in iodine content supported the concept that adaptive mechanisms tend to maintain the blood concentrations of thyroid hormone within the reference range in chronic states of high or low dietary iodine intake. However, her data were insufficient to draw conclusions about what specific concentrations of intake are healthy or detrimental.

Despite normal thyroid hormone homeostasis, it is well documented that in chronic states of iodine excess or deficiency, either state may finally lead to the development of goitre in humans and animals (Delange and Ermans,

1991; Marine, 1928; Nagataki, 1991; Roti and Vagenakis, 1991; Wollman and Breitman, 1970).

Dogs and cats have a much greater daily fluctuation of serum T_4 values than humans due to lower hormone binding in serum and shorter serum half-lives (Kaptein *et al.*, 1994). In addition, thyroid hormones may be displaced from serum-binding proteins by endogenous or exogenous inhibitors, e.g. oleic acid. There is some evidence that the intestine may provide a storage function for thyroid hormones, particularly T_3. It was found that L-thyroxine (T_4 or levothyroxine) absorption is decreased with small bowel diseases (Kaptein *et al.*, 1994). Also, a number of agents interfere with T_4 absorption after oral administration (probably the same applies to endogenous T_4), including sucralfate, ferrous sulphate, cholestyramine, colestipol, activated charcoal, aluminium hydroxide and soybean flour (Kaptein *et al.*, 1994).

In canned pet foods, there has been a trend towards lower ash content (mainly magnesium) and towards gourmet products containing more animal tissue. Iodine concentrations may be higher in gourmet cans containing marine fish or poultry products with remains of thyroid tissue (Scarlett *et al.*, 1988).

The association between the above factors and feline hyperthyroidism has not been elucidated.

As well as iodine, a xanthene dye, FD & C Red No. 3 (erythrosine), is added frequently to pet food. This artificial dye contains iodinated compounds, which releases free iodine during can thermal processing resulting in higher concentration of this element (Barbano and Bellavalle,

1984). Under conditions used in Barbano and Dellavalle's study (1984), it was found that FD & C Red No. 3 begins to release significant amounts of iodide at temperatures between 200 and 210°C and for example, when heated at 250°C for 5 min the level of free iodide was increased by more than 10-fold.

Additionally, this xanthene dye can upset thyroid hormone equilibrium by inhibition of the 5'-monodeiodinase. This latter enzyme converts T_4 in peripheral sites (e.g. liver and kidney) to biologically active T_3. Inhibition of this enzyme by FD & C Red No. 3 lowers circulating T_3 levels, which results in a compensatory increased secretion of thyroid stimulating hormone (TSH), follicular cell hypertrophy and hyperplasia, and an increased incidence of follicular cell tumours in 2-year or lifetime studies in rats (Capen, 1994 and 1997).

There is evidence that selenium plays an important role in thyroid hormone metabolism as an essential component of the three deiodinases (Arthur *et al.*, 1996). Dietary selenium concentration and the effects of its ingestion, the expression of certain enzymes connected with thyroid hormone synthesis and their relationship with feline hyperthyroidism should be investigated further.

The role of selenium can become complicated because of the widespread practice of using synthetic antioxidants in pet food manufacture (Dodds, 1995). A potent chemical antioxidant, ethoxyquin, has become recently (late 1980s) the preferred antioxidant for preserving premium commercial pet foods. Ethoxyquin's anitoxidant effect, when added to the finished product, is additive to the effects of the other two synthetic antioxidants, used for preserving animal fat sources as butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT). The synthetic antioxidants have the potential to change the bioavailability of vitamin A (essential for many biochemical

pathways including thyroid metabolism), vitamin E and selenium and may alter cellular metabolism by inducing or lowering levels of cytochrome P450, glutathione peroxidase (a selenium-dependent enzyme), and prostaglandins. By inducing cytochrome P450 and glutathione peroxidase, the levels of the reactive hydrogen peroxides and oxygen free radicals are increased and that affects the cellular metabolism. Oxidative stress can occur in the body when the balance between free radical fluxes and the antioxidant defence system is impaired. Oxidative stress plays an important role in the initiation and promotion of oncogenesis and may contribute to genetic instability and an increase in mutations in genetically predisposed individuals. The long term effects of the synthetic preservatives and their indirect influence on metabolism of thyroid hormones should be studied further.

Apart from iodine, most cat foods contain relatively high concentrations of goitrogenic compounds such as phthalates, which can be found in fish products, milk and bovine tissue (Mayer *et al.*, 1972). There are many other goitrogenic substances, e.g. polyhydroxyphenols and phenol derivatives including resorcinol, cigarette smoke, halogenated organic compounds, derivatives of 2,4-dinitrophenol (DNP); polyphenols; polycyclic aromatic hydrocarbons (PAH); azo dyes; chlorinated organic compounds (chlorinated hydrocarbons, organochlorines) with one of their subgroups polychlorinated biphenyls (PCBs); etc. (Gaitan, 1988; Roudebush, 1993), which cats may be exposed to, either through their diets (particularly fish-based meals) or in the environment, that could contribute to the development of thyroid adenomatous lesions in hyperthyroid cats. For example, chlorinated hydrocarbons tend to be chemically stable resulting in their persistence in the environment, and have solubility properties that result in their accumulation in fatty tissues. Most of these

hydrocarbons are metabolised by glucuronidation, a process which is exceptionally slow in cats. Cats, as compared to other domestic animals, are relatively deficient in their ability to conjugate xenobiotics (chemical compounds that are not produced by the animal) with glucuronic acid. Early investigations of glucuronide formation revealed a singular lack of glucuronide synthesis for phenolic substances in several organs of the cat (Dutton, 1966), and the glycine pathway is the only one operative. Cats excrete traces of glucuronide in urine but larger amounts in bile. Most of the conjugates formed by cats, biliary or urinary, are sulphates.

Glucuronidation is an important reaction for the metabolic clearance of a variety of endogenous and exogenous substances. It increases the water solubility of these compounds and thus facilitates their biliary and urinary excretion and finally their elimination from the body. The defect in glucuronide synthesis in cats has been attributed to a virtual absence of UDP (uridine diphosphate)-glucuronyltransferase activity toward certain substrates. UDP-glucuronyltransferase catalyses the actual conjunction of the endogenous or exogenous substance and glucuronic acid (Wilcke, 1984).

Functional groups that are subject to glucuronidation within the various substrates comprise aromatic as well as aliphatic hydroxyl, sulphhydryl, carboxyl and amino groups. The UGTs (UDP [uridine diphosphate]-glucuronyltransferases) represent a family of homologous enzymes with broad and overlapping substrate specificity. They are located predominantly in the endoplasmic reticulum of the liver, but significant levels of specific isoenzymes may be present in intestine, kidneys as well as other tissues (Visser, 1994). All these enzymes utilise UDP-glucuronic acid (UDPGA) as the cofactor.

If glucuronide synthesis is the major method of a particular endogenous or exogenous substance inactivation and alternate pathways are less efficient,

this substance will accumulate in the body. Glucuronidation has been recognised as a major metabolic pathway for thyroid hormone in rats, but much less is known about the importance of thyroid hormone glucuronidation in humans and nothing on this topic was available from the cat perspective. Visser *et al.* (1993) concluded that on the basis of their findings there are at least three UGT isoenzymes involved in the glucuronidation of thyroid hormones in rats.

McLain's (1989) study renewed interest in the glucuronidation of thyroid hormone, as an increasing number of drugs and xenobiotics have been found to profoundly accelerate peripheral thyroid hormone metabolism due to the induction of iodothyronine UGTs (UDP-glucuronyltransferases) activities. UGTs inducers have been shown to lower plasma levels of thyroxine by increasing its glucuronidation and elimination by the liver. The resultant compensatory increase in thyroid function may lead to thyroid hyperplasia and after chronic treatment in rats even to thyroid neoplasia (McLain, 1989). Compounds (some of these were mentioned earlier in different parts of this literature review) identified with such action include:

a) 3-methylcholanthrene (MC)-type inducers, i.e. MC itself, benzpyrene, polychlorobiphenyls (PCBs), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (dioxin), *ß*-naphtoflavone and hexachlorobenzene (HCB);
b) the anticonvulsant drugs phenobarbital and phenytoin;
c) the following hypolipidemic phenoxyisobutyrate (fibrate) derivatives: clofibrate, ciprofibrate and nafenopine. A similar mechanism of action has also been reported for other peroxisome proliferators such as dehydroepiandrosterone, polychlorinated paraffins, perfluorodecanoic acid and phtalates, as well as a number of other substances including spironolactone, the leukotriene antagonist L-649,923 and the cardiac inotropic drug OPC 8212;

d) pregnenolone-16α-carbonitrile (PCN).

Barter and Klaassen (1992) study on microsomal enzyme inducers has shown that they reduce thyroid hormone levels in rats. The above mentioned researchers were interested in the mechanism of this thyroid hormone reduction. They were considering the following possible mechanisms:

- directly by blocking the synthesis of thyroid hormones or
- indirectly by increasing the biotransformation and deactivation of thyroxine (T_4) by microsomal enzymes.

Results of their study demonstrated that reduction of thyroid hormone levels by microsomal enzyme inducers was produced in part by an extrathyroidal mechanism, quite possibly by an increase in T_4 glucuronidation. In later research Barter and Klaassen (1994) demonstrated that the microsomal enzyme inducers (phenobarbital, MC, PCB and PCN were used) increase UGTs activity toward T_4 and decrease T_4 levels. It appears that induction of UGTs plays a role in the effect of these chemicals on the thyroid gland.

Unfortunately, an extensive investigation of the thyroid hormone glucuronidation pathway and its response to microsomal enzymes inducers and a detailed mechanism of action of UDP-glucuronotransferases enzymes family (UGTs) were not a subject of this review and the reader is referred to Visser's publication (1994).

However, the differences in glucuronide synthesis from the cat perspective in consideration of thyroid hormone metabolism are not known, therefore further studies are warranted as to their potential association with feline hyperthyroidism.

Some dietary ingredients are goitrogenic too, e.g. calcium in rats on a low-iodine diet; contaminated drinking water; fluoride, rubidium, nitrate, and sulphur-containing compounds found in the drinking water (McLaren and Alexander, 1979). Similarly some factors can affect the natural availability of iodine in farm animals. The addition of lime to pasture land reduces iodine uptake, while farmyard manure can reduce the iodine content of pasture by as much as 90%. Also, some naturally occurring goitrogens such as soybean, some other beans, onion and garlic powder or oil, seaweed including kelp, gums from seaweed (carrageenan, alginates), and linseed are extensively used as ingredients in pet foods.

Soybean products have been utilised very widely as an animal protein substitute in commercial pet food since 1933. These products are obtained by subjecting soybean to variety of technological processes. Only three of twenty soybean products are produced by heating process. They were adopted in 1964 with amendment and second acceptance in 1971, in 1975 and in 1992 (AAFCO, 1993).

Middlesworth's (1957) study showed that faecal loss of thyroxine in soybean flour fed rats was almost twice that of control animals. He suggested that the goitre could result from a diminished enterohepatic recirculation and an increased faecal loss of endogenous thyroid hormone.

On the other hand, Konijn *et al.* (1973) research on goitrogenic substance from soybean flour pointed to an inhibition of iodine uptake and a decrease of its organification in the gland. It appears that the goitrogenic agent has higher effect on the formation of mono-iodotyrosine (MIT) and di-iodotyrosine (DIT) than on the production of T_3 and T_4.

Although it seems unlikely that dietary goitrogens acting alone can cause goitre, it is quite possible that their effects may be additive when

interacting with other factors, such as iodine deficiency or excess, including the day-to-day wide swings of iodine content in the diets, or other types of goitrogen from the environment (from water, air and food) and may contribute to the overall increasing incidence of human goitre in endemic areas (Gaitan, 1988; McLaren and Alexander, 1979).

There is epidemiological and experimental evidence that environmental pollutants and certain medications may cause goitre by acting directly on the thyroid gland or indirectly by altering its regulatory mechanism and/or the peripheral metabolism and excretion of thyroid hormones. These pollutants operating in genetically predisposed individuals may also trigger pathogenic mechanisms in the thyroid gland much more easily than in non-genetically prone ones (Gaitan, 1988).

All of the above hypotheses need to be clarified as to their potential association with hyperthyroidism in cats.

Chapter 3

Materials and methods

Study design

The case control study definition by Breslow and Day (1980) is as follows: "an investigation into the extent of subjects affected by a specific disease (the cases) and comparable subjects who do not have the disease (the controls) have been exposed to the disease's possible risk factors in order to evaluate the hypothesis that one or more of these is a cause of the disease". The cases and controls do not need to be representative of any population. Usually one case group and one control group is included but a second group of control animals may be added where one acceptable control group has a specific deficiency which can be overcome by the second control group.

In this study for each case a random and a matched control were selected. The use of a matched control was considered necessary, as it was relatively easy and reliable to control for cat sex and age during the analysis. A case control study based on a questionnaire was conducted over a 14-month period from 15th December 1996 to 15th February 1998.

The survey required the questionnaire to be completed by the primary care veterinarian and the owner of the cat for which a diagnosis of hyperthyroidism had been made (based on history, clinical signs and elevated concentration of thyroid hormones). For each case, the owners of two cats were recruited from client records of the participating clinic as controls and asked to fill in the questionnaire forms. Retrospective and recent cases, examined at each practice, were included in the survey.

Practices

Veterinarians from 25 veterinary practices throughout New Zealand participated in the study. The practices were located in Kerikeri (1),

Auckland (3), Hamilton (1), Taupo (1), Gisborne (1), New Plymouth (1), Wanganui (1), Marton (1), Palmerston North (3), Taradale (1), Otaki (1), Paraparaumu (1), Wellington (3), Richmond - Nelson (1), Greymouth (1), Christchurch (1), Dunedin (1), Gore (1) and Invercargill (1). Practices were included in the study when the veterinarians from that practice responded that they were willing to participate, after the project was advertised to the profession.

Cases

The case definition of hyperthyroidism for the study was based on a combination of typical history (weight loss, polyphagia, hyperactivity, polydipsia), and physical examination findings including a combination of the following signs: a palpable thyroid gland, rapid heart rate (> 200 beats/min), and weight loss. Abnormally high (above the reference range for the test performed at a laboratory) serum thyroid hormone concentrations were also required for inclusion. Animals included cases examined at the participating veterinary practices during the last seven years, up to the final date of the study. One hundred and thirty cases (C) were investigated. 125 cases were included in the study. The five deleted cases did not meet the requirements of the case definition as the serum thyroid hormone analysis had not been performed.

Controls

For each case entering the study two control cats were selected from the practice client records. One of the control cats ("matched control", MC) was matched on sex and age (± 1.5 years) for the case and the other cat ("random control", RC) was selected from practice client records using a provided selection table of random numbers. The random numbers

consisted of an initial letter which indicated the alphabetical file reference and the number of the client file within that alphabetical category. For example, random number 'J 441 2' refers to file 'J' (for, e.g. Jones) and client number '44' (or '441' if the records have over 440 J clients, or '4' if the records did not have 44). The 2 suffix refers to the client who had more than one cat. In this circumstance the second cat was surveyed. If client 'J 44' was not a cat, then the next random number was selected until a cat was identified.

The owner of each of the control cats was telephoned and, if they agreed to participate, the questionnaire was posted to them. Stamped unaddressed envelopes were provided for the postage. If an owner refused to participate, another random number was selected. Freepost addressed envelopes were provided for return of the completed questionnaires to Massey University. One hundred and twenty five matched controls and 125 random controls were involved in the study.

Data collection

Each household with cat(s) represented a specific ecological niche influenced by a complex of various sociological, biological and economic factors. The objective of the data collection was to define this system in as much detail as possible to permit a quantitative analysis.

Questionnaire

A history for the 3-year period preceding the diagnosis of hyperthyroidism for the cases and the history for the last 3-year period for controls was asked.

The questionnaire for the owners of cats with hyperthyroidism and control cats was tested with 20 cat owners to identify potential sources of misinterpretation of the questions. The questionnaire for veterinarians, for cases only, was also pilot tested with 10 veterinarians and veterinary nurses completing the questionnaire. The results of these preliminary surveys were analysed and the questionnaires were revised for the main study.

Owners of the clinical case and control cats were required to complete a fourteen-page questionnaire (see Appendix 6) which asked a range of demographic, medical, behavioural, lifestyle and feeding-related questions on their cats. Also, demographic data on cats owners was asked including their address (urban versus rural areas), moving the house, time spent at present address, type of farm if applicable, number of people in the household, people sex and age group (adult or children) and human history of thyroid diseases.

The following information on each cat was requested: breed, sex, age, hair colour and type, age at de-sexing, age at hyperthyroidism diagnosis, general medical history, clinical signs at the time of diagnosis, number of cats in the household, history of other cats with hyperthyroidism, cat's every day behaviour including fighting, potential exposures to different chemical substances used within cat's indoor and outdoor territory (like fertilisers of animal/plant origin, artificial fertilisers, herbicides, pesticides, insecticides, fungicides, pest control products and other possible chemical substances used, including the active ingredient(s). The family member who feeds the cat, frequency of feeding, feed categories and practices, other sources of food (prey, rubbish bins, neighbours), food supplements fed, observation on water intake, type of drinking water and/or milk, type of

serving dish, length of time and place of commercial food storage before and after opening the containers/package.

In addition, a 6-page questionnaire (see Appendix 7), which requested the demographic data, general medical history for the 3-year period preceding the diagnosis of hyperthyroidism, clinical signs before and at the time of the diagnosis of hyperthyroidism, was completed by the primary case veterinarian. A stamped addressed envelope was provided for return of the included questionnaires by the veterinarians and a list of names and addresses of the cases and their controls as a "check list" of participating cat owners.

Data storage

All the completed questionnaires were stored in written form and the data were entered into a computer database management system Microsoft® Access version 97 (1998). The data editing checks were conducted to screen out errors from data entry, as suggested by Rothman (1986).

Data analysis

The objective of this study was to describe potential associations between possible risk factors and occurrence of feline hyperthyroidism in New Zealand. The causal relationship may be subjective and with observational epidemiological studies, in general, it is impossible to prove the causal nature of an association (Rothman, 1986).

Breslow and Day (1980) stated that the "the basic questions to be asked in a case control study are the degree of association between risk of disease and the factors under study, the extent to which the observed associations

may result from bias, confounding and/or chance, and the extent to which they may be described as causal".

Tavris (1997) said: "It is much easier to prove that two things are associated than to show that one causes the other. Showing an association is simply a matter of conducting the proper statistical tests, but cause can never be shown by statistical tests alone. If an association with a disease is discovered which is not a cause, then getting rid of the associated factor will have no impact on preventing the disease. Whereas if that associated factor is a cause, then getting rid of it will probably be of help in preventing the disease".

The outcome variable in this feline hyperthyroidism study was the case control status of a cat. Independent (predictor) variables covered a wide range of factors of possible importance including the cat's diet and environment. Separate analyses were conducted for the comparison of cases with matched and random controls.

Matched data should be analysed using specific methods for matched analysis. Where cases and controls have been matched on a variable associated with the exposure, an analysis not taking account of matching would result in odds ratio estimates biased towards unity. On the other hand, if matching was done based on variables not associated with the exposure, a multivariable analysis accounting for matching would increase variance of the estimated parameters and consequently would be unnecessary (Schlesselman, 1982).

Breslow and Day (1980) recommend that matching should be accounted for in the analysis whenever it has been incorporated in the design.

If the matching factor is not related to disease status (it is not a confounder) matching represents overmatching, since the matching and the loss of efficiency in the required matched analysis do not increase the validity of the study (Wacholder, 1992).

Matching will improve efficiency relative to random sampling, if the matching factor is a strong risk factor, as a function of the extent to which the matching factors are differentially distributed between exposed and unexposed subjects.

For the present study standard methods of univariate analysis were used, while standard and matched methods were applied in the multivariable analysis of cases and controls.

Chapter 4

Statistical analyses

General outline of approach to data analysis

The present study of hyperthyroidism in cats in New Zealand was designed as a case control study comparing affected animals (cases) and two different control groups (matched and random). The general approach to data analysis was adapted from Hosmer and Lemeshow (1989).

In a **first** step, the data under study was briefly described to provide an overview of the study population. In the **second** stage of the epidemiological investigation, a univariate analysis was conducted assessing the association between case and control status and each of the putative risk factors separately. The results of this analysis were used as a basis for selection of a subset of significant variables to be included in the **third** step, the multivariable analysis. The first part of the multivariable logistic regression analysis was performed after separating the variables into the following groupings:

- cat and owner factors
- cat medical history
- cat indoor and outdoor environment factors
- cat diet factors and feeding practices.

The first part of the multivariable logistic regression analysis was done separately for each of the potential risk factor groupings mentioned above. The variables that remained significant ($p < 0.05$) after these analyses, on the four component models, were subjected to the second part of the multivariable logistic regression analysis. Some of the variables were excluded from this analysis either because of very low numbers within the individual categories or high numbers of missing values. Finally, the variables that remained significant at $p < 0.05$, after the second part of multivariable logistic regression analysis, together with the first order

interaction terms were subjected to the third and final part of multivariable logistic regression analysis. Stepwise unconditional logistic regression for the case-random control group and stepwise conditional logistic regression for the case-matched controls, were applied to identify the most important factors within the risk factor groupings.

The following statistical methods were used in each of the three analytical steps in this data analysis.

Analysis of the initial step consisted of a **descriptive analysis** of the data, including the use of graphical methods. In step number two, a **univariate analysis** of each variable using a univariate logistic regression model was performed to screen the main dataset statistically for variables which were significantly associated with cat's case-random control status, based on the score test statistic at a significance level $p < 0.1$ (Hosmer and Lemeshow, 1989). In logistic regression the parameters of the model are estimated using the maximum-likelihood method, i.e. the coefficients that make the observed results most "likely" are selected. A screening criterion of 0.1 for case-random controls status was chosen to ensure that all potentially important variables were included in the next analytical step, the **multivariable analysis**. A few variables which were significant in the univariate analysis, but had questionable biological interpretability or were strongly collinear with other variables already included, or could not clearly be considered as potential causal risk factors rather than outcomes, were excluded from the multivariable analysis. Factors which remained statistically significant at $p < 0.05$ after the first phase of multivariable analysis performed on four component models, were subjected to the second part of multivariable analysis. Finally, the remaining significant variables, together with any significant first order interaction terms, were

included in the final multivariable analysis. Variables which were statistically significant at a p value less than 0.05, but were not included in the final multivariable analysis, are described in more detail using tables.

Step number three consisted of two analytical approaches which were used to develop multivariable models for the two separate comparisons in the study. Method one used a **forward stepwise unconditional logistic regression** (Norušis, 1994) approach to fit a multivariable model comparing cases with random controls and method two used a **forward stepwise conditional logistic regression** (Norušis, 1994b) approach to fit a multivariable model for the cases and matched controls.

Continuous variables included in the univariate and multivariable models were transformed into categorical variables.

Methods used in the multivariable analysis

A stepwise selection procedure uses a statistical algorithm to decide on the inclusion of variables into a model. During each step the variable which produces the greatest change in the log-likelihood relative to the previous model is sequentially included in the model until the p value of the likelihood ratio chi-square test exceeds a predetermined value. At every step a check for elimination of variables already included in the model is also performed, their continued importance being determined by using the likelihood ratio test at a given significance value (Hosmer and Lemeshow, 1989). The first order interaction terms were entered into the model using a stepwise approach after a main effects model had been obtained.

Forward stepwise multiple unconditional logistic regression

Forward stepwise unconditional logistic regression was used for the cases and random controls. All the independent variables which had a probability $p < 0.1$ in the univariate analysis or were considered potentially biologically important were included. 95% confidence intervals (CI) for all variables in this multivariable analysis were used and the first category of each variable was the reference category. All two-category variables were coded as "0" and "1" to indicate "absence" and "presence" of something. This is called dummy-variable or indicator-variable coding. For the continuous variables or variables with more than two categories the new indicator variables were created to represent the categories. In this case the coefficients for the new variables represent the effect of each category compared to a reference category. The coefficient for the first category is "0" and it is treated in this study as the reference category. The "Indicator" type of contrast was chosen as this contrast indicates the absence or presence of category membership. The reference category was represented in the contrast matrix as a row of zeros.

Forward stepwise multiple conditional logistic regression

Conditional logistic regression models are designed for situations in which one or more cases, which show the response of interest, are matched with one or more controls, which do not show the response. The most common situation involves 1-1 matching, though 1-N and M-N matching is also seen.

Forward stepwise conditional logistic regression was used to investigate the relationship between case-control status and a set of putative factors for matched data. As in the previous analysis, 95% confidence intervals (CI)

were estimated for the effects of all variables, and the first category of each variable ("0") was used as the reference category.

There is only a limited number of statistical packages which have specific procedures for conducting conditional logistic regression. If such a procedure is not available, conditional logistic regression can be performed using the discrete logistic model by forming a stratum for each matched set and then running the Cox regression model. In this study each case had only one matched control, so that the likelihood function for the conditional logistic regression reduces to that of the Cox model for a continuous scale (Anon. SAS/STAT' Software; Stokes *et al.*, 1995).

The procedure described by Nichols (1998) was used to implement conditional logistic regression model in SPSS™ version 8 (SPSS Inc., Chicago, U.S.A.) using the COXREG procedure. The dependent variable was coded 1 for the cases and 2 for the matched controls. The technical requirement is that the case in each set has a positive value that is smaller than that for its control. This is required so that the probability of being a case is modelled.

Odds ratios, p-values and 95% confidence intervals around the OR were presented, for all main effects if they were significant ($p < 0.05$) and their interactions, in the final model.

All univariate and multivariable regression analyses were carried out using the logistic and Cox regression procedures in SPSS™ (SPSS Inc., Chicago, U.S.A.).

In addition to the above analyses, the following analyses were performed.

Cox regression

Cox regression models (proportional hazards models) are similar to standard regression models where a prediction of a dependent variable (length of time until occurrence of an event) is presented as a function of a set of independent variables. However, unlike ordinary regression models, Cox regression models can be used when there are censored observations.

Cox regression (COXREG) was used to analyse the effect of various risk factors on age of diagnosis based on data from cases and random controls. The latter were treated as censored observations (those for which the event, "hyperthyroidism", has not yet occurred). The survival and hazard curves for the risk factors included in the final model, are presented.

Kaplan-Meier survival analysis

Special techniques are needed for analysis of data that contain censored observations. Since these techniques are often used to analyse data in which the event of interest is death, they are known as survival time or failure time techniques.

Kaplan-Meier survival analysis (Kleinbaum, 1996) is very closely related to the actuarial estimates method, where the period of time under the study is subdivided into intervals for which the various probabilities are estimated. However, to compute Kaplan-Meier estimates of the probability of being event-free at various time points, there is no need to establish intervals at which the various probabilities are evaluated. Instead, the estimation of the probability of an event is calculated each time an event is being observed. To determine Kaplan-Meier estimates of the survival curve, the evaluation of the survival curve at each of the time points at

which an event occurs, i.e. at each of the uncensored time points, is performed.

This survival analysis technique provides the mean survival time. That time is not the average of the observed survival times, since it does not make sense to compute the usual arithmetic average if not all observations experience the outcome such as death or onset of disease. Special techniques are used to estimate mean survival time when there are censored observations.

Kaplan-Meier survival analysis (KM) was used to compare the different options of treatment, including medical, surgical, radioactive iodine (I^{131}) treatment and no treatment at all, on survival time (months) of hyperthyroid cats from diagnosis until the end of the study. In this analysis, the censored cases are those for which the event, "death", did not occur during the study period. The presented survival function plots allow a graphical interpretation of the data for different types of treatment.

All of the statistical analyses and the estimates of all the survival and hazard curves mentioned above were calculated and presented graphically using the SPSS statistical software.

All SPSS codes (syntax) used for different analyses are presented in Appendix 8.

Chapter 5

Results

Descriptive analyses

One hundred and twenty five cases, 125 case-matched controls and 125 random controls were involved in the study between December 1996 and February 1998. Tables 1, 2 and 3 show the sex, age and breed profiles respectively of the cases and control cats. There were differences in the age, sex and breed distributions of cases compared with the random controls. The proportion of speyed females was higher than that of castrated males. Cats older than 12 years of age were predominantly affected. The proportion of purebred cats was lower than domestic short and long haired cats.

Table 1. Sex profiles of cases, matched and random control groups

	Questionnaire type			Total
	Cases	Matched controls	Random controls	
Male castrated	49	49	68	166
Female speyed	76	73	52	201
Male entire			3	3
Female entire		3	2	5
Total	125	125	125	375

Table 2. Age profiles of cases, matched and random control groups

	Questionnaire type			Total
	Cases	Matched controls	Random controls	
5 months to 9 years	4	3	80	87
9 to 12 years	20	30	30	80
12 to 19.5 years	101	92	15	208
Total	125	125	125	375

Table 3. Breed profiles of cases, matched and random control groups

	Questionnaire type			Total
	Cases	Matched controls	Random controls	
Domestic short hair	93	80	87	260
Domestic long hair	26	21	20	67
Siamese	5	11	2	18
Persian		4	5	9
Burmese		6	2	8
Other pure breeds	1	3	9	13
Total	125	125	125	375

Cats' age distribution at time of hyperthyroidism diagnosis

Figure 1 shows the age distribution of cats at the time of hyperthyroidism diagnosis for 125 cats. The youngest cat was diagnosed at 7 years 1 month of age (85 months), the oldest at 18 years 10 months of age (226 months).

The mean of cats' age distribution at the time of hyperthyroidism diagnosis is 13 years 1 month (157 months). It is in agreement with the average age at the time of hyperthyroidism diagnosis for cats from all studies conducted so far (Broussard *et al.*, 1995; Holzworth *et al.*, 1980; Jones and Johnstone, 1981; Labuc and Jones, 1986 and 1988; Scarlett, 1994; Scarlett *et al.*, 1988; Peterson, 1984 and 1998; Peterson *et al.*, 1979, 1983 and 1994; Thoday, 1988; Thoday and Mooney, 1992).

As the standard deviation is 27.81, it gives an age range for two-thirds of observations from 128 months (10 years 8 months) to 185 months (15 years 5 months). While 95% of hyperthyroid cats will be included between 101 months (8 years 5 months) and 212 months (17 years 8 months) of age.

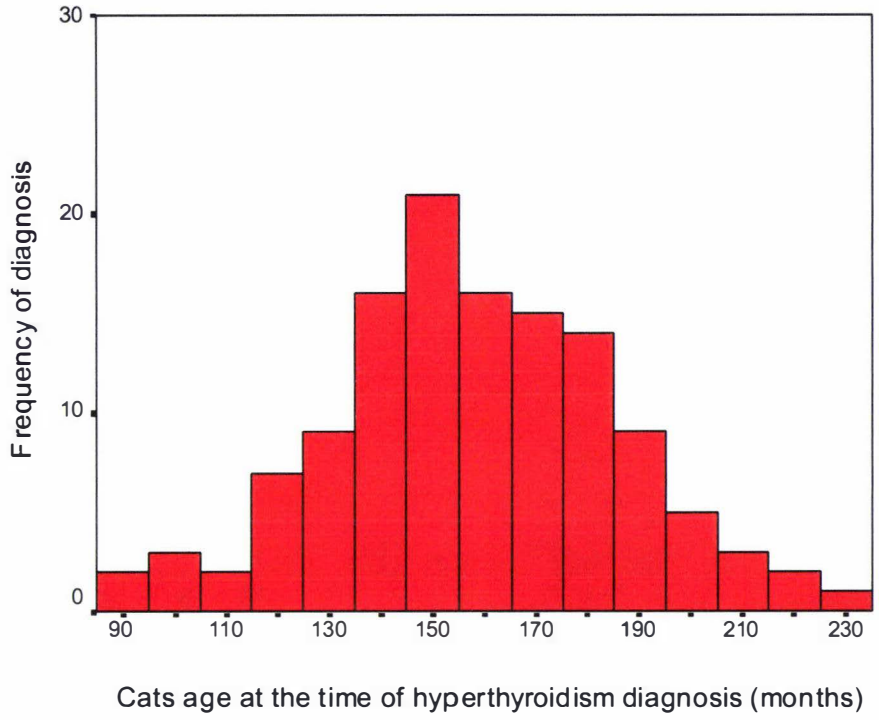


Figure 1. Age distribution at time of hyperthyroidism diagnosis (months) (125 cases; mean = 157 months; median = 155 months; standard deviation = 27.81)

Monthly diagnosis of all cases throughout the study

Figure 2 (shown in discussion part) presents a histogram of the monthly pattern of diagnosis of all cases throughout the study. It is obvious that the diagnosis of hyperthyroidism is more common during the six warmer months in New Zealand, from October until March, with the exception of January.

Clinical signs frequency in hyperthyroid cats

The percentage of historical and clinical findings in affected cases was analysed. The figures are presented in Table 8 (shown in discussion part). Most of them are in agreement with five previously published summaries (Broussard and Peterson, 1993; Broussard *et al*, 1995; Hoenig *et al*., 1982; Holzworth *et al*., 1980; Peterson *et al*., 1983; Thoday and Mooney, 1992).

Univariate logistic regression analyses of the case-random control comparison

Cat owner factors

The results of the univariate analyses of the main data set are listed in Tables 9a to 9j (Appendix 1). The significant explanatory variables ($p < 0.1$) from the random control comparison included:

- moving house (together with the cat) within the last 5-year period
- living longer than 3 years at the present address
- having a human (most affected people were females) in the household with a history of any thyroid gland diseases
- length of ownership of the affected cat of more than 3 years
- cat having more than one owner (the difference of six or more months between cat's age and length of cat's ownership was assumed to signify that the cat had had more than one owner)
- "unknown" or "stressful" origin (wild, stray, found abandoned, given for adoption, from S.P.C.A. [Society for the Prevention of Cruelty to Animals], etc.)
- having two or more children in the household (this was protective).

Cat factors including medical history

The significant explanatory variables ($p < 0.1$) from the random control testing included:

- age
- breed (this was protective for Siamese)

- breeding history for pure breeds (this was protective for cats bred overseas)
- sex (this was protective for male cats)
- age at desexing
- regular vaccination against calicivirus, herpesvirus and panleukopenia (this was protective)
- history of vaccination against feline leukaemia virus (this was protective)
- any dental, respiratory, urinary and gastrointestinal tract diseases
- episodes of diarrhoea and starvation in cat's medical history.

Cat's indoor and outdoor environment

The significant explanatory variables ($p < 0.1$) from the random control comparison included:

- presence of pasture and industrial area in cat's territory (both protective factors)
- more than 3 hour per day spent outside (this was protective)
- sleeping predominantly on the floor, wool carpets and woollen clothes/fabrics or sheepskin
- using washing as a preferred method for cleaning cat's bedding
- using sawdust for bedding or litter trays
- having other hyperthyroid cat(s) in the household (especially if these cats were related to the hyperthyroid cat)
- having a pet rabbit in the household (this was protective)
- lighter than normal weight
- lazy or very lazy behaviour
- occasional to frequent fights (this was protective)

- regular exposure to pesticides/fungicides used on pot plants at home
- addition of chemicals to vases with cut flowers in order to prolong flower life (this was protective)
- anti-flea products (particularly long acting ones) used regularly on cat's bed/bedding
- regular flea control at home using mainly long acting anti-flea products
- in the case of owners having a farm or livestock, using mineral licks for farm animals (this was protective).

Cat's diet and feeding practices

The significant explanatory variables ($p < 0.1$) from the case-random control status included:

- food alternation on weekly basis
- eating commercial canned food (if half to all of cat's daily food requirements was from this source it was associated with increased risk for developing hyperthyroidism)
- eating raw meat (in particular beef meat, mince, fat, offal and fish)
- eating dairy products such as cream, ice cream and cheese
- eating cooked beef, lamb, mutton, pork, chicken and fish (as human leftovers)
- eating eggs
- adding medicines, yeast, vitamins and minerals including kelp to cat food
- excessive intake of daily fluids
- cleaning the cat's serving dish more than once a day
- using a microwave oven to warm or defrost cat's meals.

All of the earlier mentioned variables were connected with higher risk for developing hyperthyroidism.

On the other hand, the following factors from the cat's diet were protective ones:

- eating commercial cat dry food. The protection effect increases as the amount of dry food daily intake increases.
- other sources of food such as small rodents and birds
- drinking rain water
- drinking water from a bath.

Forward stepwise multiple unconditional logistic regression analyses of the case-random control comparison

The first part of the multivariable logistic regression analysis was performed separately for each of the four potential risk factor groupings mentioned earlier, representing owner and cat factors, cat medical history, cat environment factors and cat diet factors. The variables that remained significant ($p < 0.05$) after these analyses were subjected to the second part of the multivariable logistic regression analysis. Finally, the variables that remained significant at $p < 0.05$, after the second part of multivariable logistic regression analysis, together with the first order interaction terms were subjected to the third - final part of multivariable logistic regression analysis.

Tables 10a and 10b (Appendix 2) list the statistics for the significant variables found by forward stepwise multiple unconditional logistic regression analysis of the case-random control comparison after the second part of this analysis (Norušis, 1994). Only the variables marked "*" in Tables 9a to 9j (Appendix 1) were subjected to multivariable logistic

regression analysis. The rest of the significant variables (from the univariate analysis) were excluded from that analysis either because of very low numbers within the categories or a high number of missing values, had questionable validity or were strongly collinear with other variables already included, or could not clearly be considered as potential causal risk factors rather than outcomes. It was found that the proportions of different types of food, the brand names and the flavours of commercial food, and the types of raw or cooked meat were subject to big error (human memory) and it was very difficult to assess how genuine the data was.

The variable "cat weight" (marked "#" in Tables 10b and 11a, Appendices 2 and 3) was significant for both comparisons (case-random and case-matched) but was excluded from both final models because it could not clearly be considered a risk factor as opposed to an outcome variable.

Final model for case-random control comparison

The final model for case-random control comparison (Table 4) includes main effects terms for the:

- age (older cats are more likely to develop hyperthyroidism)
- breed (this was protective for Siamese)
- sex (females are three times as likely to have the condition as males)
- cat's age at desexing (the category "don't know", which indicates either that the cat had another owner or unknown cat's origin, was associated with increased risk for developing hyperthyroidism)
- history of any dental diseases/infections including oral cavity diseases (although this effect was controlled for cat age, occurrence of the dental

disorders was associated with a five and half – fold higher risk of developing hyperthyroidism)

- sleeping predominantly on the floor, which may be a carpeted surface (99.2% of New Zealand houses are lined with carpet; this study data)
- anti-flea products used regularly on cat's bed/bedding
- eating half or more of daily food requirement as canned commercial cat food was associated with twice the risk of developing hyperthyroidism.

No interaction terms were significant in this model.

Table 4. Final multivariable forward stepwise unconditional logistic regression model based on all case and random control data, showing level of significance (p) for the variables and their categories, odds ratios (OR) and 95% confidence intervals around the OR.

Variables	p	OR (Exp(B))	95% CI for Exp(B)	
			Lower	Upper
Cat age	0.0000			
5 mths to 9 years		1.0		
9 to 12 years	0.0009	13.8120	2.9305	65.0981
> 12 years	0.0000	520.4563	83.0956	3259.7973
Cat breed	0.0193			
DSH		1.0		
DLH	0.7701	1.1930	0.3655	3.8941
Siamese	0.0019	0.0124	0.0008	0.1990
Other pure breeds	0.9474	1.1164	0.0423	29.4948
Cat sex				
Male		1.0		
Female	0.0222	3.2700	1.1848	9.0245
Cat age at desexing	0.0092			
Up 6 or at 6 months		1.0		
> 6 months	0.1220	0.4055	0.1292	1.2728
Don't know	0.0573	4.1381	0.9569	17.8948
Dental diseases				
No		1.0		
Yes	0.0039	5.5000	1.7295	17.4903
Bedding - floor				
No		1.0		
Yes	0.0038	6.6386	1.8444	23.8946
Anti-flea products used regularly on cat's bed/bedding				
No		1.0		
Yes	0.0035	57.5677	3.7926	873.8087
Daily can food proportion	0.0369			
None		1.0		
Up to 1/2	0.4649	0.5093	0.0834	3.1116
> 1/2 to all	0.4445	2.0510	0.3254	12.9281

Residual Chi Squared = 7.294 with 8 df Sig = 0.5052

Forward stepwise multiple conditional logistic regression analyses of the case-matched control comparison

Forward stepwise conditional logistic regression was used to investigate the relationship between case-control status and a set of putative factors for matched data. As in the previous analysis, 95% confidence intervals (CI) were estimated for the effects of all variables, and the first category of each variable ("0") was used as the reference category.

In this study each case had only one matched control, so that the likelihood function for the conditional logistic regression reduces to that of the Cox model for a continuous scale (Anon, SAS/STAT® Software; Stokes *et al.*, 1995).

The procedure described by Nichols (1998) was used to implement conditional logistic regression model in SPSS™ version 8 (SPSS Inc., Chicago, U.S.A.) using the COXREG procedure.

Odds ratios, p-value and 95% confidence intervals around the OR were presented, for all main effects if they were significant ($p < 0.05$) and their interactions, in the final model.

The significant variables identified from the case-matched control comparison (Tables 11a and 11b in Appendix 3), by way of forward stepwise multiple conditional logistic regression after the first part of this analysis (Norušis, 1994b), were:

- age at desexing
- episodes of cat fight/bite abscesses and diarrhoea in cat's medical history
- number of other cats in the household (this was protective if more than one cat was present in the household)

- cat's body weight
- regular usage of manure (animal/plant origin fertilisers, organic fertilisers) on cat's outdoor territory
- regular usage of fly sprays on cat's indoor territory
- eating commercial cat canned food (acting as the protective factor if fed up to a half of cat's daily food requirement)
- eating a variety of flavours of commercial canned cat food
- eating dairy products such as cheese, ice cream, cream, yoghurt and butter
- eating vegetables, rice and fruits (dinner leftovers) (this was a protective factor)
- drinking an excessive quantity of fluids
- drinking water from puddles
- regular milk drinking.

Final model for case-matched control comparison

Table 5 shows the final model computed by stepwise forward conditional logistic regression of the case-matched control data set. The final model included main effects terms for:

- history of diarrhoea (the cats with episodes of diarrhoea were seven times as likely to develop hyperthyroidism)
- number of other cats in the household (this was protective if more than one cat was present in the household)
- regular usage of fly sprays on cat's indoor territory
- eating a variety of flavours of commercial canned cat food
- interaction between drinking water from puddles and the regular use of manure (animal/plant origin fertilisers) on cat's outdoor territory.

Drinking water from puddles was not of much importance *per se* (OR=1.5744, 95% CI=0.6779-3.6562), but drinking water from puddles if regular use of manure on cat's outdoor territory was reported became important (OR=5.2572, 95% CI=1.0794-25.6052).

Table 12 (Appendix 4) lists all the variables which were found significant ($p < 0.1$) when subjected to the univariate analyses for case (1) versus. random control (0). These variables were not included in the multivariable logistic regression analyses because of a very high number of missing observations or very low number of observations, or in case of "death" to avoid cause-result effect.

Tables 13a to 13g (Appendix 5) record all the variables which were found not significant when subjected to the univariate analyses from cases, random and matched control cats.

Table 5. Final multivarible forward stepwise conditional logistic regression model based on all case and matched control data, showing level of significance (p) for the variables, their categories and interaction, odds ratios (OR) and 95% confidence intervals around the OR.

Variables	p	OR (Exp(B))	95% CI for Exp(B)	
			Lower	Upper
Main effects terms				
Diarrhoea				
No		1.0		
Yes	0.0005	7.3547	2.4045	22.4953
No. of other cats in house	0.0015			
0		1.0		
One	0.0128	0.3757	0.1738	0.8120
Two or more	0.0005	0.1516	0.0525	0.4381
Regular use of fly sprays on cat's indoor territory				
No		1.0		
Yes	0.0219	3.3246	1.1900	9.2877
Feeding a variety of can food flavours				
No		1.0		
Yes	0.0048	3.8004	1.5026	9.6121
Regular use of manure on cat's territory				
No		1.0		
Yes	0.1179	2.0771	0.8309	5.1924
Drinks water from puddles				
No		1.0		
Yes	0.2911	1.5744	0.6779	3.6562
Interaction terms				
Drinks water from puddles x regular use of manure on cat's outdoor territory	0.0399	5.2572	1.0794	25.6052

Residual Chi Squared = 5.83 with 9 df Sig = 0.7568

Cox regression (proportional hazards model) for case-random status

Further to the above analyses, Cox regression - proportional hazards model (Norušis, 1994b) was used to analyse the effect of various risk factors on age of diagnosis (cat survival time expressed in months) based on data from cases and random controls. Some of the requested survival and hazard curves for the risk factors present in the final case-random model are shown below (Figure 2 to Figure 11). The most pronounced hazard effects were for cat breed (protective for Siamese), desexing at "don't know" cat age, sleeping predominantly on the floor, use of anti-flea treatment on cat's bed/bedding and feeding the cat with more than a half of daily food proportion as commercial canned food.

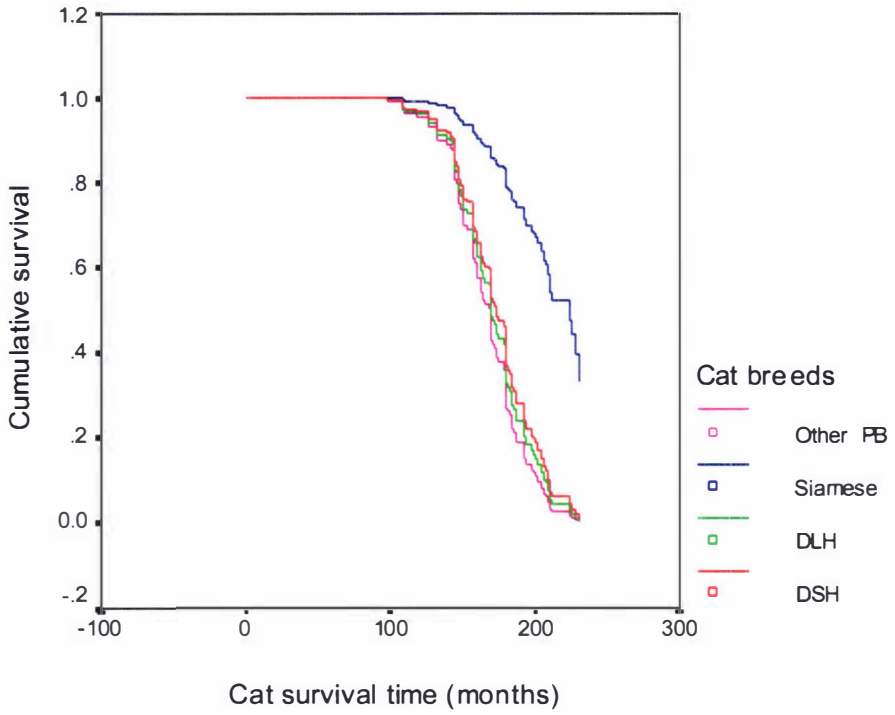


Figure 2. Cumulative survival functions for four breeds of cats from case-random comparison (Other PB = other pure breeds; DLH = domestic long hair; DSH = domestic short hair)

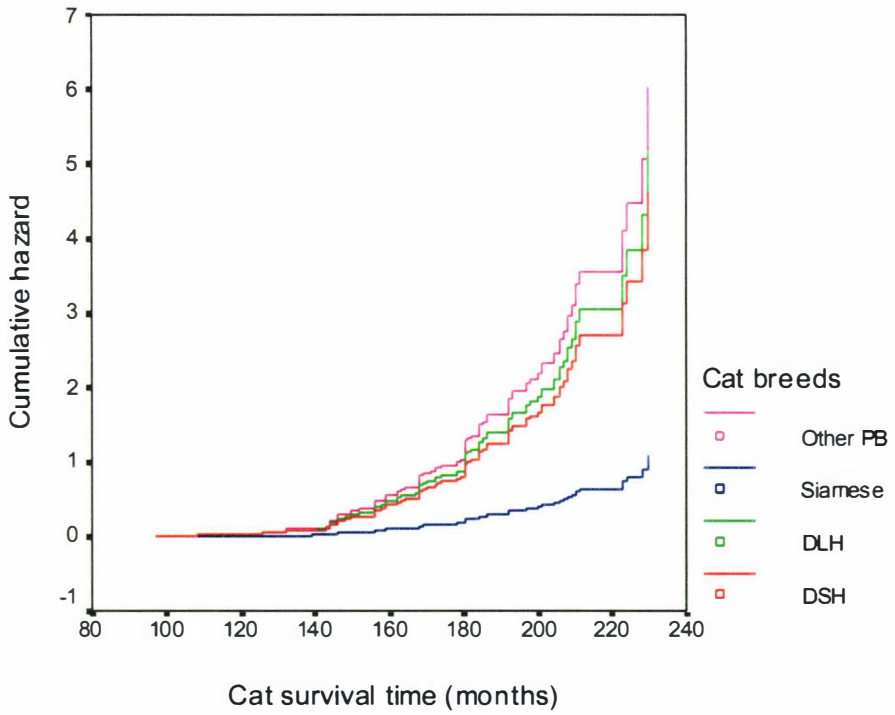


Figure 3. Cumulative hazard functions for four breeds of cats from case-random comparison (Other PB = other pure breeds; DLH = domestic long hair; DSH = domestic short hair)

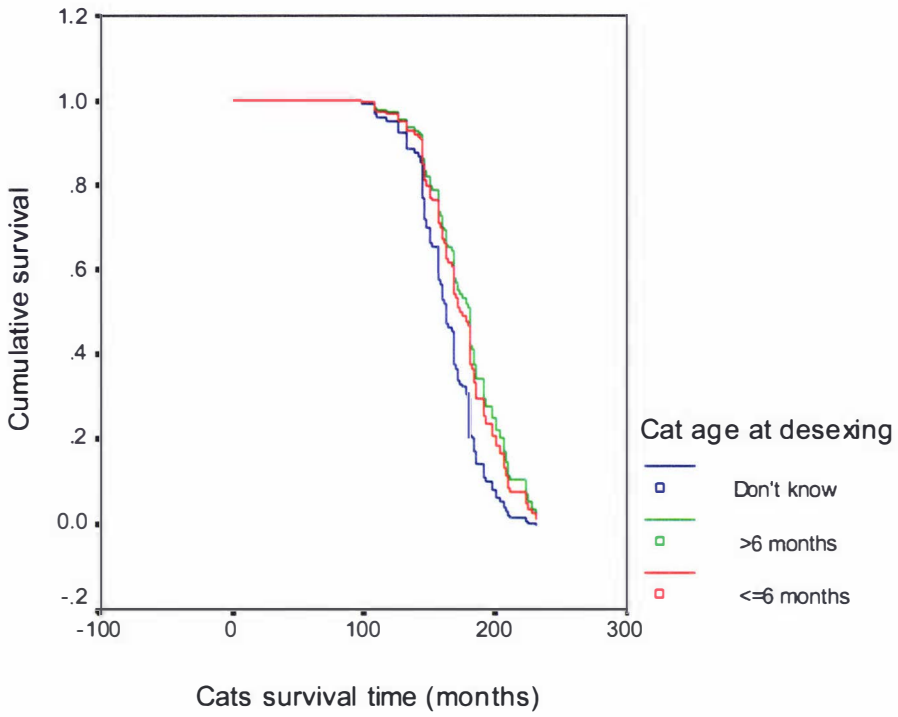


Figure 4. Cumulative survival functions for three categories of cat age at desexing for cats from case-random comparison

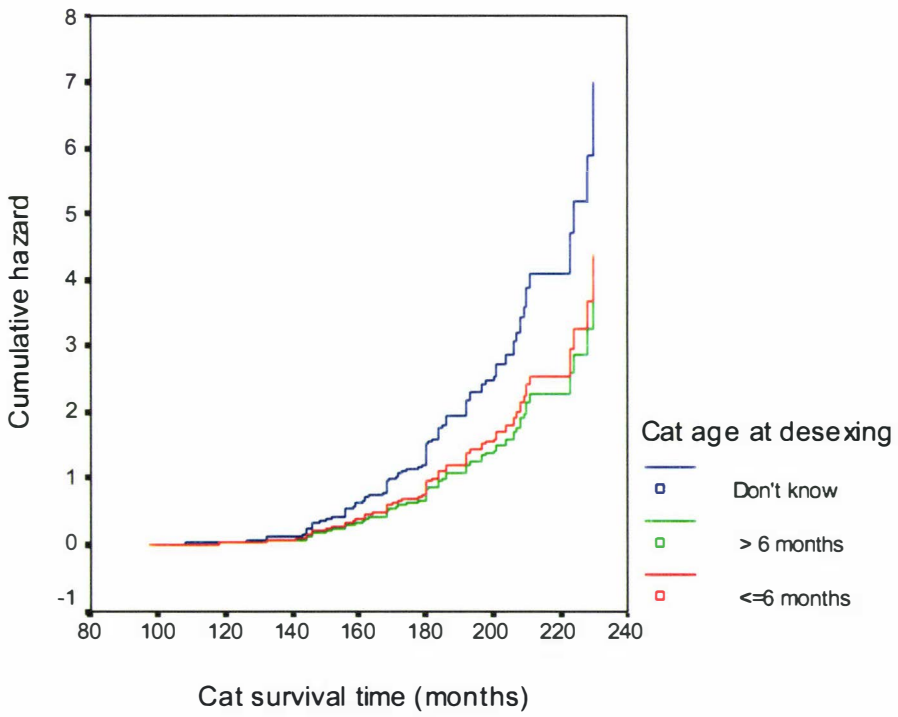


Figure 5. Cumulative hazard functions for three categories of cat age at desexing for cats from case-random comparison

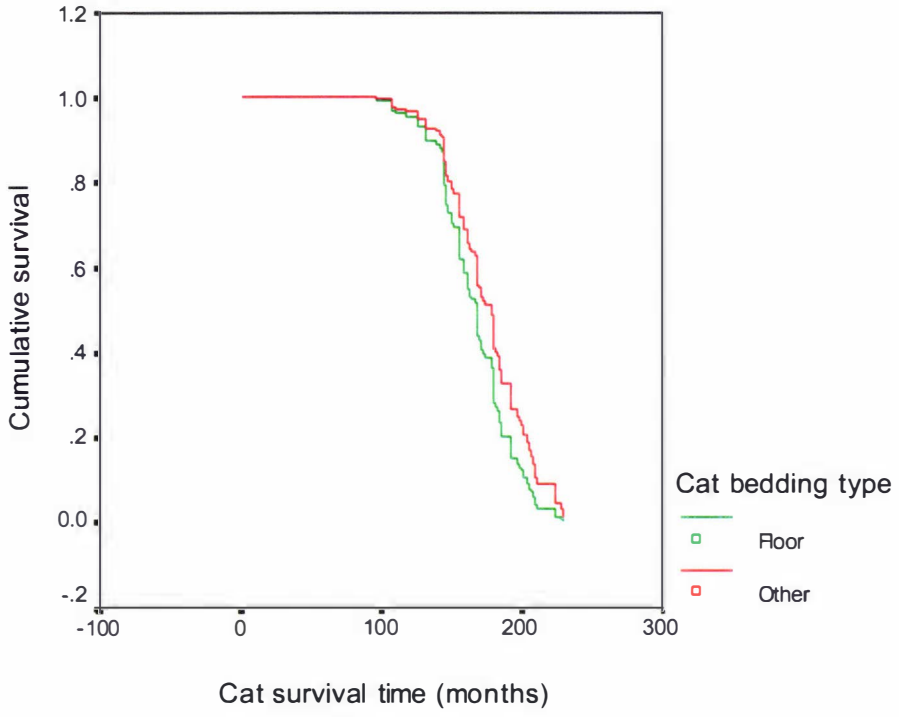


Figure 6. Cumulative survival functions for two categories of cats bedding for cats from case-random comparison

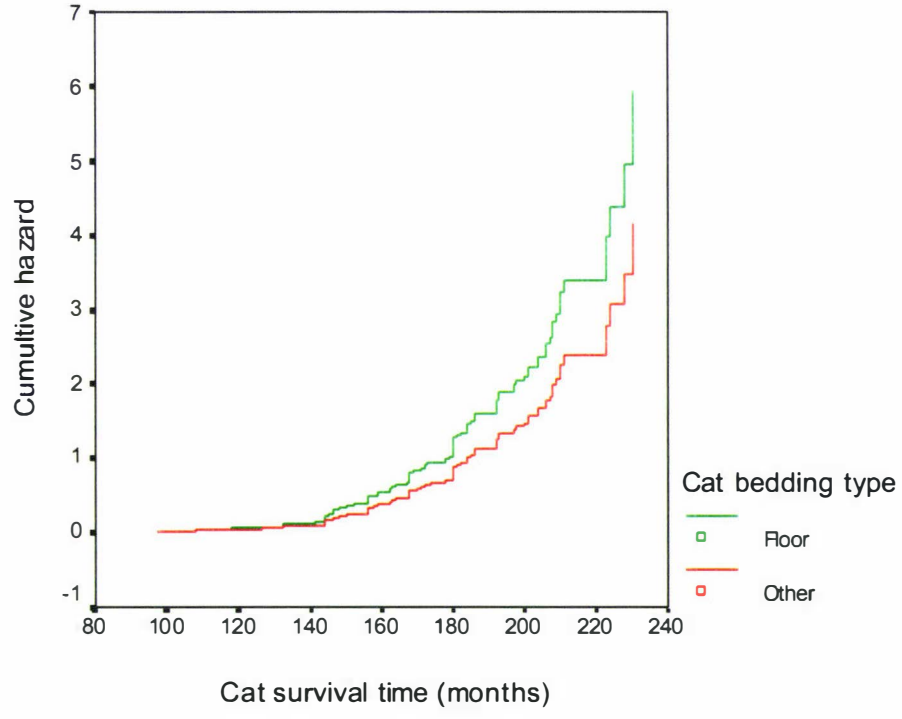


Figure 7. Cumulative hazard functions for two categories of cats bedding for cats from case-random comparison

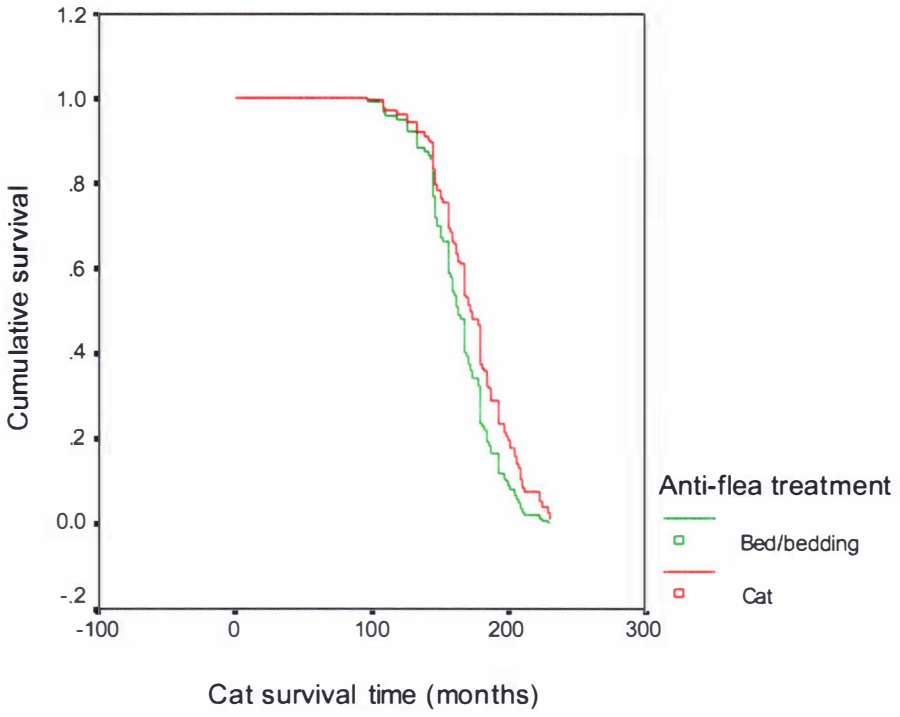


Figure 8. Cumulative survival functions for two places where anti-flea treatment is applied for cats from case-random comparison

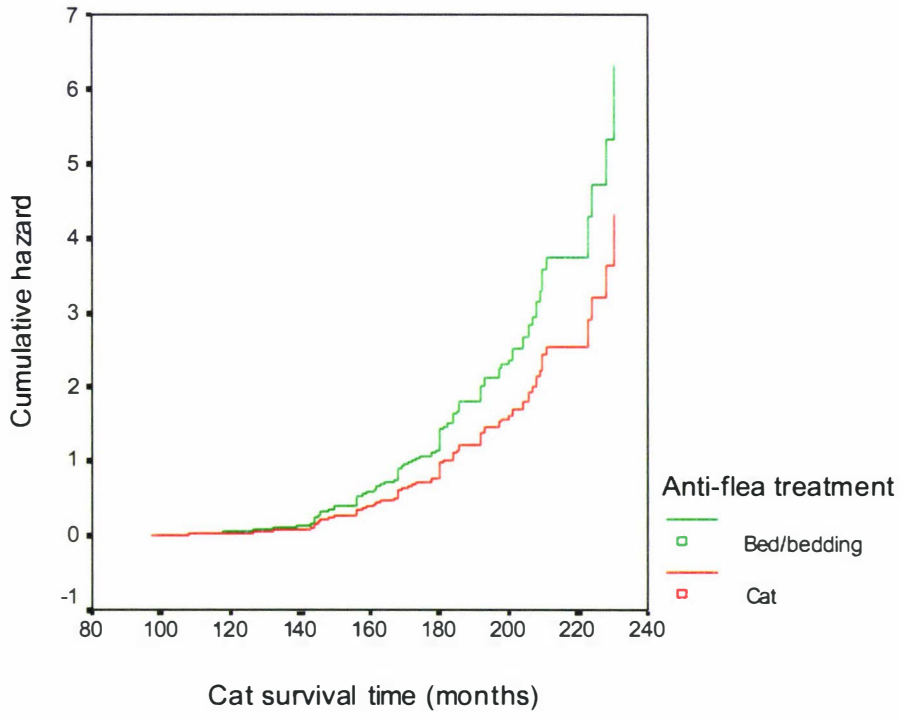


Figure 9. Cumulative hazard functions for two places where anti-flea treatment is applied for cats from case-random comparison

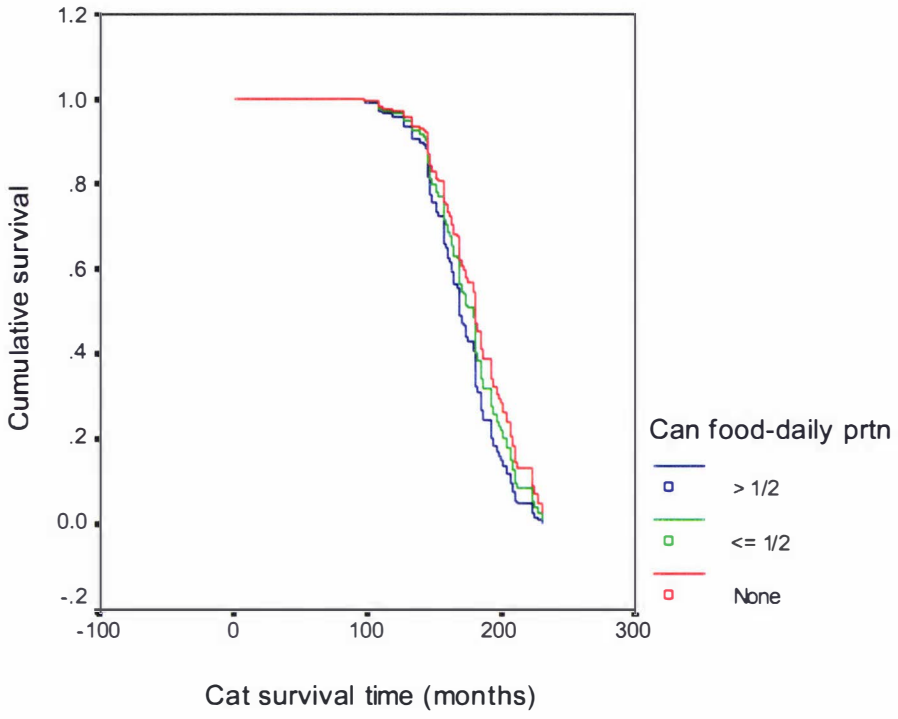


Figure 10. Cumulative survival functions for daily proportions of commercial cat canned food for cats from case-random comparison (prtn = proportion)

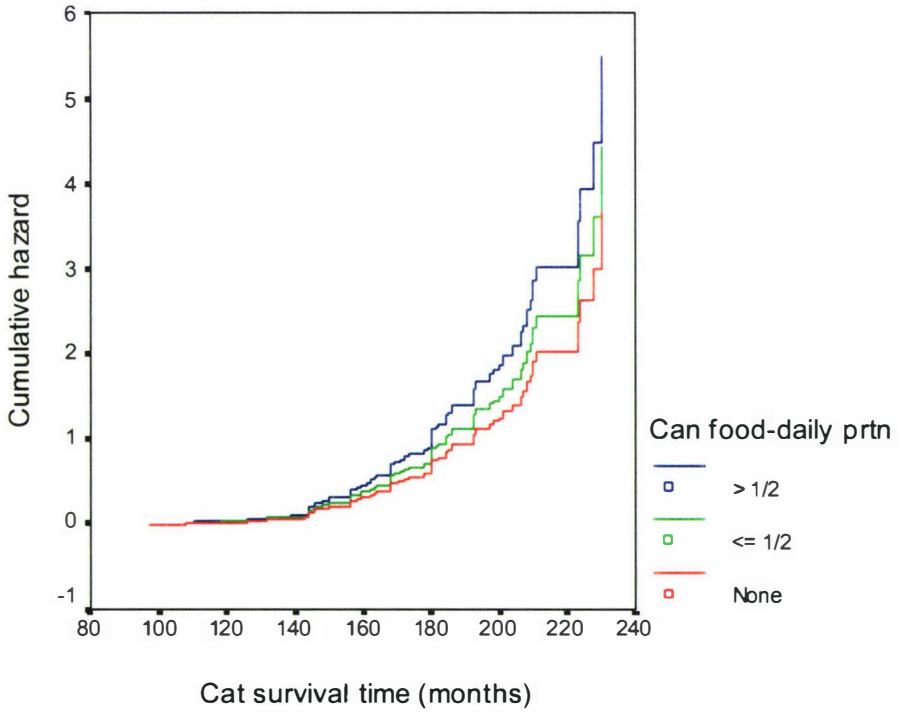


Figure 11. Cumulative hazard functions for daily proportions of commercial cat canned food for cats from case-random comparison (prtn = proportion)

Kaplan-Meier survival analysis of hyperthyroid cats subjected to different methods of treatment

Additionally, Kaplan-Meier (Kleinbaum, 1996) survival analysis for cases status was performed to compare the different methods of treatment on survival time (months) of hyperthyroid cats from initial diagnosis up to the final date of the study. There were 19 deaths among hyperthyroid cats, four in matched controls, but only one in the random control group.

Four survival curves were compared:

- Survival of hyperthyroid cats from the date of diagnosis in cases where the cats did not receive any treatment at all (5.6% [7 cases, 3 deaths]. Figure 12).
- Survival of hyperthyroid cats from initial diagnosis when the cats were subjected to medical treatment (Figure 13). In total, medical treatment accounted for 58.4% of hyperthyroid treatment (73 cases, 10 deaths). Carbimazole (5 mg tablets; Neo-mercazole, Nicholas/Roche) was used in 94.5% (69 cases), saturated potassium iodide solution in 4.1% (3 cases), while PTU (50 mg propylthiouracil; Abbott) in 1.4% (1 case) of medically treated cases. In 34.2% of medically treated cases, this treatment was also used to stabilise the patient before thyroidectomy (12.3%, 9 cases) or radioactive iodine treatment (21.9%, 16 cases).
- Survival of hyperthyroid cats since the date of diagnosis in cases where thyroidectomy (unilateral or bilateral) was performed (9.6% [12 cases, 1 death], Figure 14).
- Survival of hyperthyroid cats since the date of diagnosis when the cats were treated with radioactive iodine I^{131} (45.6% [57 cases, 5 deaths]. Figure 15).

All curves are step-like because the proportion of the cats surviving changes precisely at the point when a subject dies. It is noticeable that cats subjected to any kind of treatment survived longer in contrast to cats not treated at all. The differences between the types of treatment on survival could be explained by the numbers of cats exposed to different treatments and the different approaches to hyperthyroid cat treatment, and presence or absence of any concurrent non thyroidal diseases at the time of diagnosis of hyperthyroidism. For example, older cats with symptoms of renal failure are more likely to be treated with medical treatment than radioactive iodine or surgery, and automatically they face a shorter survival time.

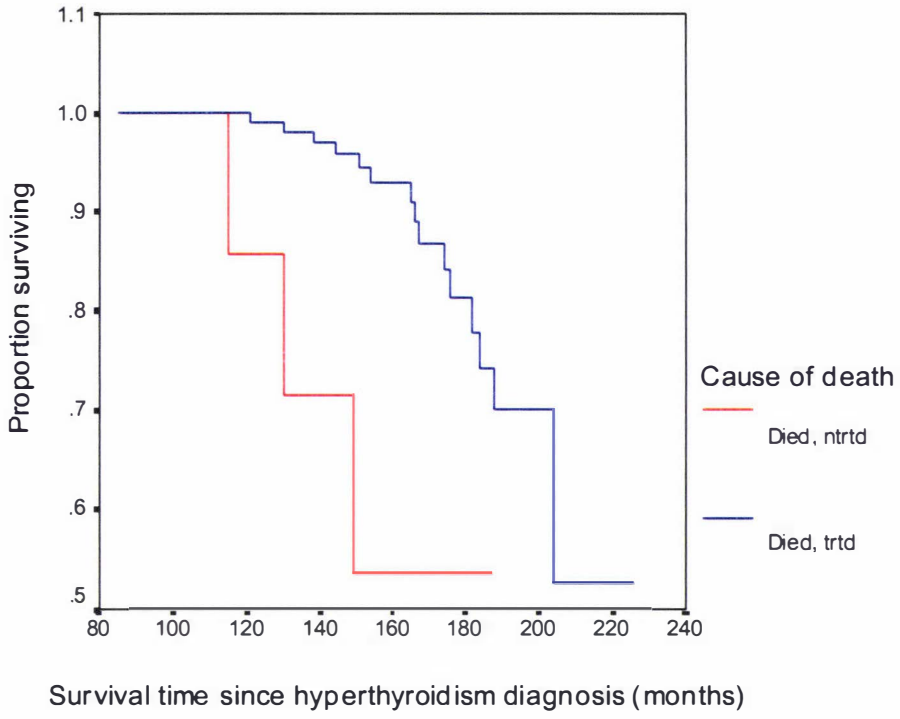


Figure 12. Kaplan-Meier survival curve for hyperthyroid cats treated with any method (trtd) and not treated at all (ntrtd) (125 cases)

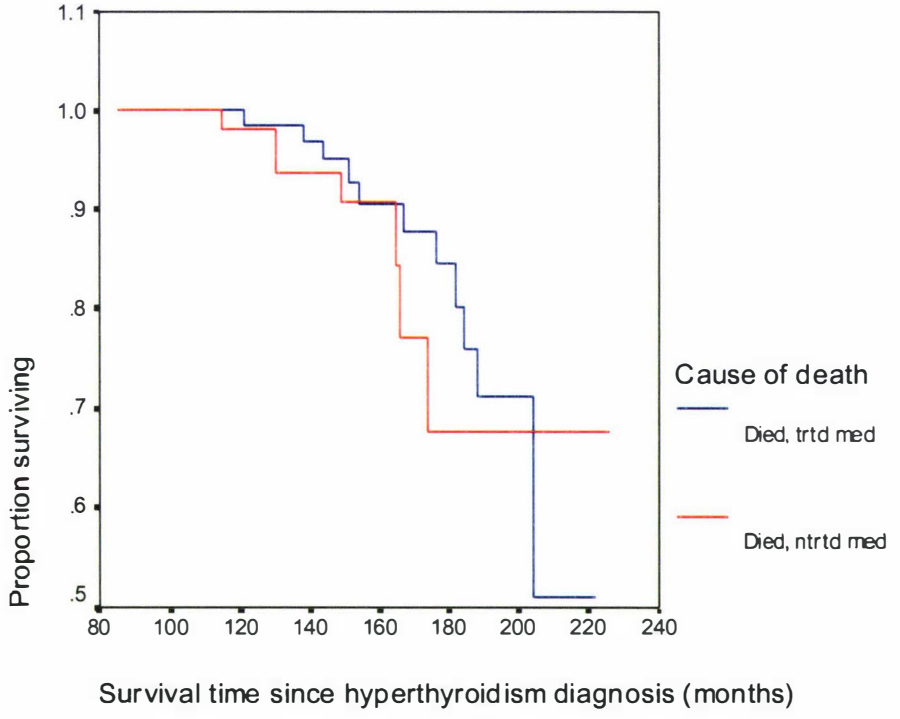


Figure 13. Kaplan-Meier survival curve for hyperthyroid cats treated (trtd med) and not treated (ntrtd med) medically (125 cases)

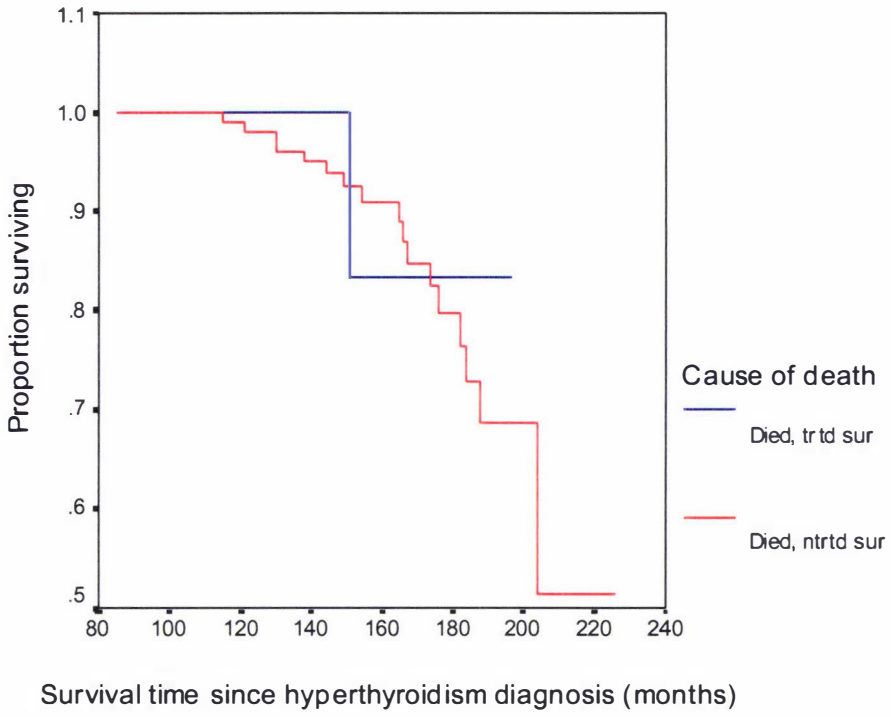


Figure 14. Kaplan-Meier survival curve for hyperthyroid cats treated (trtd sur) and not treated (ntrtd sur) surgically (125 cases)

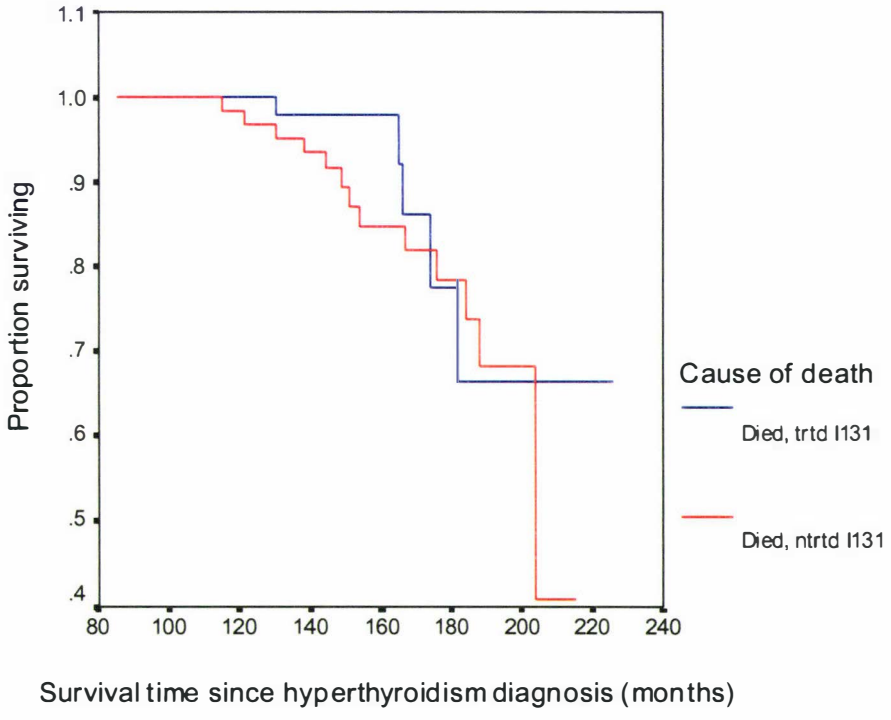


Figure 15. Kaplan-Meier survival curve for hyperthyroid cats treated (trtd I131) and not treated (ntrtd I131) with radioactive iodine (I^{131}) (125 cases)

Chapter 6

General discussion

Introduction to general discussion

This thesis reports on an observational epidemiological study into the risk factors for feline hyperthyroidism in New Zealand.

The results have confirmed some of the findings of previously published work (Kass *et al.*, 1998; Scarlett, 1994; Scarlett *et al.*, 1988) and suggested new avenues for further research. However, like previous reports, this study was unsuccessful in isolating one single dominant factor which could be incriminated in the development of the disease, and adds weight to the view that this is a multifactorial disease.

In contrast to two previous studies conducted in the United States, in which only case-matched controls were researched (matched by sex and age [± 1 year in the study of Scarlett *et al.* (1988) and ± 3 years in that of Kass *et al.* (1998)]), in the New Zealand study two control populations were used. One of the control cats ("matched control") was matched on sex and age (± 1.5 years) for the case and the other cat ("random control") was selected from practice records using a selection table of random numbers.
These two different control groups were included for comparison with hyperthyroid cats in order to limit potential bias. The use of the two comparison groups made it possible to differentiate risk factors for feline hyperthyroidism specifically, as opposed to risk factors for aged cats in general. Variables that were significantly associated with hyperthyroidism in both comparisons without any confounding effect were considered particularly good candidates to be true risk factors for developing the disease.

The final models for both data sets (Tables 4 and 5, shown in the Results) were different, having in common only two variables. The first variable was linked by two kinds of environmental exposures having in common the use of insecticides: "anti-flea products used regularly on cat's bed/bedding" and "regular use of fly sprays on cat's indoor territory". The second variable was combined from two closely related dietary factors: "daily proportion of commercial canned food" and "feeding the cat with a variety of flavours of commercial canned food".

The lack of common variables between the two analytical approaches could have resulted from at last three causes. Firstly, the matched analysis focuses on external influences, while the random analysis focuses more on internal influences (age, breed, sex, etc.). Secondly, the number of animals in the study did not provide sufficient statistical power to ensure that all relevant variables were in the final model, although these were more in common at the immediately preceding stage of the analysis. Thirdly, some variables could be measured only crudely further reducing power.

However, before the final models were produced, multivariable analyses were performed for both comparisons on four separate groups of variables, i.e. cat and owner factors, the cat's medical history, indoor and outdoor environment, and diet. The outcomes of those pre final analyses are presented in Tables 10a and 10b (Appendix 2) for case random control animals and in Tables 11a and 11b (Appendix 3) for cases versus matched controls. It is noticeable that another five potential risk factors were common for both comparisons and they were characterised by very similar odds ratios. They were as follows: age at desexing, cat body weight, the proportion of commercial canned food fed daily (with different odds ratios), the daily quantity of fluid drunk and two variables having in

common insecticides (anti-flea products used regularly on cat's bed and regular use of fly sprays on cat's indoor territory).

To avoid confusion, the explanation of the potential risk factors for developing feline hyperthyroidism will be discussed, with the two final models together, and will be divided into four main groups representing cat and owner factors, the medical history, environmental factors and dietary factors.

Cat and owner factors

Cat age

The models suggested that feline hyperthyroidism affects older cats. Figure 1 (shown in the Results) shows the mean of cats' age distribution at the time of hyperthyroidism diagnosis is 13 years 1 month (157 months), which is in agreement with the average age at the time of the diagnosis of hyperthyroidism for cats from all studies conducted so far (Broussard *et al.*, 1995; Holzworth *et al.*, 1980; Jones and Johnstone, 1981; Labuc and Jones, 1986 and 1988; Scarlett, 1994; Scarlett *et al.*, 1988; Peterson 1984 and 1998; Peterson *et al.*, 1979, 1983 and 1994; Thoday, 1988; Thoday and Mooney, 1992).

Feline hyperthyroidism resembles one form of human thyrotoxicosis – Plummer's disease which predominantly affects older people with long-standing goitres (Brownlie and Wells, 1990; Croxson, 1997; Studer and Gerber, 1991). Human toxic nodular goitre is a slow, insidiously developing process in which iodine exposure may precipitate thyrotoxicosis. A common finding in human multinodular goitre is subclinical hyperthyroidism, i.e. suppressed TSH secretion unresponsive to thyrotropin-releasing hormone (TRH), in the presence of normal serum thyroxine and triiodothyronine

levels, which often precedes the appearance of overt hyperthyroidism (Studer and Gerber, 1991a). It can be assumed that similar processes are occurring in pre-hyperthyroid cats' thyroid glands but there has not been any research on pre-clinical evaluation of feline hyperthyroidism from the serum thyroid hormones values or thyroid gland histology.

Although it was thought that toxic nodular goitre did not occur in children; in fact it does and has been diagnosed in young human adults aged 10 to 15 years (Studer *et al.*, 1985). This is again in agreement with the feline condition, where sporadic cases are reported as early as 4 years of age.

Cat breed

The persistent protective effect of breed for Siamese cats was found in all three studies conducted so far. In addition, Kass *et al.* (1998) found that Himalayan cats, which are genetically related to Siamese, have a diminished risk for developing hyperthyroidism. The Himalayan breed is not a common breed in New Zealand and these cats were under-represented in this study. Both Scarlett *et al.* (1988) and the results of this study, confirmed that Siamese cats have a 10-fold lower likelihood for developing the disease. Also, a lower prevalence in pure breed cats has been noticed in the United States (Kass *et al.*, 1998; Scarlett *et al.*, 1988) and United Kingdom (Blaxter and Gruffydd-Jones, 1994). This study may provide evidence for genetic factor(s) associated with this condition or indicate a lack of exposure to the aetiologic agent(s), or just reflect differences in the life expectancy of pedigree cats compared to mixed breed cats. However, from personal observations, it would seem that old Siamese cats are presented to veterinarians much more frequently than other purebred cats. This observation was also confirmed by personal communication with a number of veterinary practitioners in New Zealand and overseas.

Cat sex

This New Zealand study, in contrast to previous reports (Kass *et al.*, 1998; Scarlett, 1994 and Scarlett *et al.*, 1988), found that female cats had 3.3 times the risk of hyperthyroidism compared to male cats. All of the hyperthyroid cats in this study were ovariohysterectomised or castrated.

This new finding is in agreement with the human incidence of thyroid disorders (Studer *et al.*, 1985). As with most other thyroid diseases in people, the incidence of toxic nodular goitre is three to five times higher in females than in males (Studer *et al.*, 1985). This human ratio is also well duplicated in this study (Table 7) and in Brownlie and Wells'(1990) report about thyrotoxicosis in North Canterbury, New Zealand.

There are at least two possible explanations for the observation that human females (and maybe female cats) are more likely to be affected by thyroid disorders than males. The first explanation of this phenomenon is that it could be due to inheritance. Whether the inheritance is dominant, or recessive (as in humans), has not been determined. The reason for the human female predominance in autoimmune thyroid disease and other organ-specific autoimmune diseases is not known. Evidence shows that genes on the X or Y chromosome may influence responses to immunoregulatory genes, with one gene modifying another (Volpé, 1991).

Secondly, the underlying assumption for the fact stated above seems to be that sex hormones can affect immunoregulatory mechanisms (Volpé, 1991). This additional factor (cat sex) could be considered in further investigations of the aetiology of feline hyperthyroidism.

Cat age at desexing

Another variable was "cat age at desexing" where category "don't know" elevated hyperthyroid risk four times compared to categories "6 months of age or less" and "more than 6 months of age".

There is not a clear explanation for the relationship between "unknown age" at desexing and hyperthyroidism. The unknown age of the cat when it was neutered, on the other hand, may simply be an indicator that cats have had more than one owner and are more likely to originate from unknown sources; and because of that these cats might have been exposed to constant or intermittent stress levels (including starvation) at some stage of their lives or may be they are just older than thought. The following two variables, "number of cat owners" and "origin of the cat", were significant in univariate case-random control analysis (Table 9a, Appendix 1) but they disappeared in the multivariable case-random control model. As evidence of a cat having had more than one owner, a difference of six or more months between cat's age and length of cat's ownership was assumed to signify that the cat had had more than one owner.

Additionally, differences between sexes in the effect of starvation on thyroid hormones were shown in the work of Visser *et al.* (1996). It is known that glucuronidation is a major pathway of thyroid hormone metabolism in rats, involving at least three different hepatic UGTs (UDP [uridine diphosphate]-glucuronyltransferases): bilirubin UGT, phenol UGT and androsterone UGT. Visser *et al.* (1996) studied the effects of short-term (3 days) fasting and long-term (3 weeks) food restriction to one-third of normal intake on hepatic UGT activities for thyroxine (T_4), triiodothyronine (T_3), bilirubin and androsterone in male and female Wistar rats with either a functional (high activity) or a defective (low activity)

androsterone UGT gene. It is known that food deprivation can induce centrally mediated hypothyroidism in rats and because of it, the results of Visser *et al.* (1996) study were compared with the results obtained in methimazole (MMI - anti-thyroid agent)-induced hypothyroid rats. Both, short-term and long-term fasting, produced largely parallel increases in T_4 and bilirubin UGT activities. These effects were greater in males than in females, and were reproduced in MMI-treated rats. Visser *et al.* (1996) results demonstrated different sex-dependent effects of food deprivation on hepatic T_4 and T_3 glucuronidation that are associated with changes in the expression of bilirubin UGT and androsterone UGT, respectively. For the increased T_4 and bilirubin UGT activities at least, these effects appear to be maintained by the hypothyroid state of the (semi)starved animals. This finding can explain partially, not only the differences between sexes but also the above finding where cats with "unknown" or "stressful" origin (wild, stray, found abandoned, given for adoption, from S.P.C.A., etc.) were found to be more at risk of developing hyperthyroidism than cats from "known" or "not stressful" origin (bred in private homes or by breeders).

Cat medical history

Diseases

In spite of the suggestion that there may exist an infectious agent (Scarlett *et al.*, 1988; Taylor *et al.*, 1989) which could serve as a potential risk factor for the development of feline hyperthyroidism, none of the previous studies conducted identify any specific risk associated with particular infectious or other diseases.

From this study performed in New Zealand, the possible risk factors that remained statistically significant in multivariable models after controlling for age and sex were "history of any dental/oral cavity diseases" (case-

random controls model) and "episodes of diarrhoea" (case-matched control comparison) in the last 3-year period preceding diagnosis of hyperthyroidism.

Dental and oral cavity diseases

Although the "dental diseases" variable was controlled for cat age, the occurrence of dental disorders was associated with a five-and-half-fold higher risk of developing hyperthyroidism. The preliminary findings of a survey on all diseases of cats which was conducted in the United States (Anon, 1996) suggest that, in younger cats (0 to 7 years, n = 9148, where n = number of observations), oral diseases as a whole were the most common abnormality (9.9%) but less common than a diagnosis of 'healthy' (34.2%). However, by seven to 10 years of age (n=1795), oral cavity diseases, at 20.1%, displace healthy (18.9%) as the most common disease category, while in age group from 10 to 25 years (n=2981) oral diseases are at 19.5% in comparison to 11.9% regarded as healthy. Dental diseases are well known to be a problem in the cat and they may or may not involve other body systems, and could be associated with disturbances of the immune system.

Diarrhoea

Incidents of diarrhoea were associated with a 7.4-fold higher risk of developing hyperthyroidism. This association could be disputed taking into account that diarrhoea is a common sign (20% reported by cat owners versus 6% reported by veterinarians, Table 8) at the time of diagnosis of feline hyperthyroidism. It was very difficult to establish how cat owners were able to distinguish the occurrence of diarrhoea in the following time frame: (i) a three year period preceding the diagnosis of hyperthyroidism,

(ii) the immediate period preceding the diagnosis, and (iii) the period during which diagnosis was made. A thorough analysis of diarrhoea as a risk factor for feline hyperthyroidism requires a different methodology such as a cohort study and/or attempts to isolate potential infectious agent(s) or dietary factors. Unfortunately this was not possible to do with the present data, and was beyond the scope of this project.

The possibility of immunological cross-reactions between infectious agent(s) and thyroid antigens should be taken into account also. Studies by Luo *et al.* (1993 and 1994) showed that immunisation of mice with the enterobacterium *Yersinia enterocolitica* leads to the production of antibodies against the human TSH receptor (TSHR). These results suggested that molecular mimicry, between *Y. enterocolitica* envelope proteins and the TSHR, might play a role in the induction of autoantibodies to TSHR as develops in humans Graves' disease.

However, diarrhoea does not appear to be part of the 'top 10 feline diagnoses' by age category in any age group (Anon. 1996) and therefore this may just turn out to be one of a number of 'side effects' of hyperthyroidism. Diarrhoea also was a significant variable in univariate analysis of case-random animals (Table 9c, Appendix 1).

On top of those diseases mentioned above, several other ones were identified in earlier analyses. For example, Scarlett *et al.* (1988) showed there was one possible health risk factor, from the univariate screening, 'previous urinary infections'. This risk factor was also significant in the univariate case-random controls analysis in this New Zealand study together with respiratory and gastrointestinal tract diseases, and incidents of diarrhoea and starvation (Tables 9b and 9c, Appendix 1). In case-random pre-final multivariable comparison this New Zealand study confirmed again associations between feline hyperthyroidism and respiratory and

urinary tract diseases and episodes of starvation (Table 10a, Appendix 2), while in case-matched comparison - "cat bite/fight abscesses" was a significant factor (Table 11a, Appendix 3).

The increased risk of developing hyperthyroidism in the presence of cat fight/bite abscesses could be linked to changes of hyperthyroid cats' behaviour. Hyperactivity and aggressiveness/irritability are encountered in 31% and 33% respectively of hyperthyroid cats, as reported by New Zealand cat owners (Table 8). This could lead to more frequent fighting with other cats in the house or neighbourhood. Fighting cats presumably spend time outside and they are obviously more likely to meet other cats with infectious diseases. This latter finding will be discussed in more detail in the cat indoor and outdoor environmental section of the discussion.

The study of Jones *et al.* (1995) on prevalence of feline immunodeficiency virus infection in hyperthyroid cats did not support the involvement of that virus in the pathogenesis of feline hyperthyroidism.

Preventative medicine

Another interesting finding coupled with the cats' health status was from the univariate case-random comparison. It indicated the protective effect of calicivirus, herpesvirus and panleukopenia regular vaccination (annually in New Zealand) and also feline leukaemia virus vaccination (FeLV) (Table 9b, Appendix 1). This is reflected in protective significance for various answers to the following questions: (i) "How many times has the cat been to the vet over the last 3 years and on how many occasions was this for vaccination?"; (ii) "Do you vaccinate your cat against calicivirus, herpesvirus and panleukopenia, against chlamydia, and against feline leukaemia virus (FeLV)?"; (iii) "The recent results of feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) tests" (Table 12, Appendix 4). However all those variables, except the calicivirus,

herpesvirus and panleukopenia vaccination (with "No" and "Yes" categories), were not submitted to multivariable analysis because of high number of missing observations. The random control animals were more likely to be subjected to non routine vaccinations (chlamydia and FeLV) and screening tests against relatively recently described diseases such as FeLV and FIV than the cats from matched controls, so these facts could increase the potential bias. On the other hand, the vaccination simply could be a marker for cats which visit the veterinarian at least once a year and in general these cats receive better care but are also therefore more likely to be found to have enlarged thyroid gland(s), so the protective effect is interesting.

Indoor and outdoor environment

Multicat households

The results from univariate case-random controls analysis will be discussed first.

The hyperthyroid cats in this New Zealand study were more likely to have another hyperthyroid cat in the household than random or matched control cats (Table 6; Tables 9d and 9e, Appendix 1). Often the other hyperthyroid cat was related to the cat under the survey suggesting a possible genetic predisposition. From Table 6 we see that the other hyperthyroid cat numbers were low, as well as the health information on the cat's relatives was unavailable in most cases, so this finding should be treated with caution. None of these multicat hyperthyroid households had a human member with a thyroid gland disorder.

Table 6. Other hyperthyroid cats profiles allocated to households of cases, matched and random control groups; their blood relationship to hyperthyroid cat under survey and number of cats in that household

No. of cats in house	Blood relationship of other cat(s) to cat under survey		Questionnaire type			Total
			Cases	Matched controls	Random controls	
Two	Not related	Other hyperthyroid cat	2	1		3
		Total	2	1		3
	Related	Other hyperthyroid cat	1			1
		Total	1			1
Three or more	Not related	Other hyperthyroid cat	2	2	1	5
		Total	2	2	1	5
	Related	Other hyperthyroid cat	2			2
		Total	2			2

Another very interesting finding was that the households with hyperthyroid cat were more likely to have a human (most affected people were females) with a history of a thyroid gland disorder, see Table 7.

Table 7. Human thyroid disorders profiles allocated to households of cases, matched and random control cat groups

Gender	Thyroid condition	Questionnaire type			Total
		Cases	Matched controls	Random controls	
Female	Hyperthyroidism	4	2		6
	Hypothyroidism	1	1	1	3
	Total	5	3	1	9
Male	Hyperthyroidism			1	1
	Hypothyroidism	1			1
	Total	1		1	2

This study revealed 11 persons reporting malfunctioning thyroid glands, nine of them were females, two males. Five of those females and one male were from households with a hyperthyroid cat, three females from matched

controls and one of each, female and male, from random controls type households. The total figure of 11 from the sample of 349 households taking part in the study is higher than the national average. In New Zealand, for example in North Canterbury, the annual incidence of thyrotoxicosis was 25.8 per 100 000 (female 40.7, male 10.5) in a 3-year (1983-1985) epidemiological study (Brownlie and Wells, 1990).

The higher number of people reporting thyroid disorders from the hyperthyroid cats households could indicate environmental factor(s) or even infectious agent(s). The potential infectious agent could be transmissible from cats to people or vice versa. In Brownlie and Wells' report (1990) the proportion of patients (7%) with a history of any acute infection (flu-like, acute bronchitis, gastro-enteritis, urinary tract infection) prior to diagnosis did not seem to be more than one would expect in the community. The variable 'infective agent(s)' and often long duration of symptoms before diagnosis makes the identification of possible environmental factors very difficult.

Returning to cats, in final multivariable case-matched comparison, the density of cats in a household was acting as a protective factor if more than one cat was present in the household (Table 5). The findings in this study are not compatible with the hypothesis of an infectious agent as a risk factor.

Luttikhuis (1989) and Thoday (1988) reported hyperthyroidism in a queen and her two male offspring, and in two female siblings. This familial prevalence could indicate the existence of an inherited predisposition to this disorder. Since these cats were raised in the same households, and had common exposure to risk factor(s), thus the possibility of horizontal

transmission of some unidentified infectious agent(s) could not be excluded.

94.4% of hyperthyroid cats in this New Zealand study lived in homes in which there were no other hyperthyroid cats, 46.4% of hyperthyroid cats were from one cat households, 72.8% of hyperthyroid cats spent more than 3 hours outside (those more likely to meet other cats with any infectious diseases). The last finding was associated with decreased risk of developing hyperthyroidism in univariate case-random analysis (Table 9d, Appendix 1) and it is in agreement with Scarlett *et al.* (1988) who found an 11-fold increase in risk in predominantly indoor cats compared with predominantly outdoor cats. The above mentioned "time spent outside" figure (> 3 hours per day outside) for hyperthyroid cats is between the figures for cats from matched (61.6%) and random (82.4%) controls. The "one cat households" were less common among the controls (30.4% in matched and 40.0% in random controls) than in cases and "other hyperthyroid cat(s) in household" was more likely to be true in the case group than in the controls (Table 6).

Floor as cat bedding

One of the potential risk factors from the cat indoor environment was the floor being the cat's bed (final case-random model, Table 4).

Firstly, it should be said that some of the clinical signs of hyperthyroidism, like heat intolerance, intermittent increase of the body temperature or inability to control the body temperature may force a cat to seek the coolest place in the house, which may be the floor.

Secondly, from this study it appears that 99.2% of New Zealand houses are carpeted mainly with 100% sheep wool carpets. The wool which is used for making those carpets is from New Zealand sheep fleece. Because of the

nature of the New Zealand climate, intensive animal production, and genetic make up of the animals, sheep are prone to fly strike, lice infestations and gastrointestinal parasitism. The first two conditions are treated with organophosphates, synthetic pyrethroids or insect growth regulators on a regular basis all year around. Unfortunately, all of those chemical substances can leave residues in the fleece. The dip residue value in New Zealand fine wools is on average 30-35 ppm (parts per million). International standards permit only up to 5 ppm (Anon, 1998). However much of this should be removed in processing. Apart from the chemical residues, the dyes used for wool dying, anti-static and anti-microbial carpet treatments, protection against soil, oil-based and water-based stains (e.g. Scotchgard™, E-Gard, Ibergard) and deodorising, should be considered also.

The floor, as a main type of cat's bedding, was associated with 6.6-fold higher risk of developing hyperthyroidism and it was supported by findings from univariate analysis (Table 9d, Appendix 1), where other wool products were indicated as a potential risk factors for hyperthyroidism. They were sheepskin and woollen articles (old blankets, pieces of old wool carpets, old wool garments) used for a cat bed or bedding.

Another potential risk factor from the univariate case-random analysis was sawdust which was used for bedding or litter trays (Table 9d, Appendix 1). However, the use of cat litter *per se* was not a risk factor, in contrast to the findings of Kass *et al.* (1998). The use of litter could be just an indication of the different lifestyle of cats in New Zealand which are predominantly outdoor as opposed to cats in U.S.A., which have a predominantly indoor environment.

Regular use of anti-flea products on cat's bed/bedding and regular use of fly sprays at home

The regular use of anti-flea products on the cat's bedding and regular use of fly sprays at home were identified as potential risk factors for developing hyperthyroidism from the multivariable final case-random and case-matched comparisons, respectively.

Natural pyrethrins, synthetic pyrethroids and insect growth regulators are the main active ingredients of anti-flea products and also fly sprays. These first two active ingredients are postulated to be relatively non toxic. Pyrethrins have low mammalian toxicity and no toxic effects have been observed in cats and dogs treated with pyrethrins or pyrethrin-containing products at recommended dose rates. Insecticides with natural pyrethrins and synthetic pyrethroids listed as the main components would be more frequently used than the others. The regular use of anti-flea products at home and on the cat were significant only in univariate case-random analysis (Tables 9e and 9f, Appendix 1). Together, the cat's exposure to fly sprays, flea control products used regularly at home and anti-flea products, used on cats or cat's bed/bedding, could have an additive and augmented thyrotoxic effects on the animal's thyroid function, and this could be increased additionally by the cat's licking habits. This study (univariate analysis) indicated that long acting (effective for more then seven days to up to ten months) anti-flea products used both at home and on the cat and/or cat's bed/bedding were associated with a much higher risk of developing hyperthyroidism than the use of shorter acting products (effective for up to 7 days).

The regular exposure of cats to commercial topical ectoparasitides was associated with increased risk of developing hyperthyroidism in the study of Scarlett *et al.* (1988) (multivariable analysis) and in the investigations of Kass *et al.* (1998) (univariate analysis). Both studies were not able to

identify a specific commercial anti-flea product or an ingredient connected with the risk.

The formulation of these ectoparasiticide preparations should be taken into account not only from the point of view of their active ingredients but also from the other chemicals added to the formulations, e.g. flowing, binding or propellant agents.

Keeping in mind that it is extremely difficult to obtain an accurate and detailed history of flea control over a cat's lifetime, any weighting the role played by flea and fly control products, and the residues in New Zealand wool carpets on the development of hyperthyroidism must be questioned.

Regular use of animal and plant origin fertilisers (manure/organic fertilisers) on cat's outdoor territory and drinking water from puddles

Exposure to some environmental chemicals (e.g. pesticides, herbicides) is known to induce thyroid abnormalities in other species (Florsheim *et al.*, 1963; Porter *et al.*, 1993; Singh H. and Singh T.P., 1980) and could play a role in the pathogenesis of feline hyperthyroidism.

Artificial fertilisers, pesticides, and herbicides applied regularly to the cat's environment have previously been associated with an increased risk of developing hyperthyroidism (Scarlett *et al.*, 1988). From this study it appeared that the regular use of animal and plant origin fertilisers (manure) on a cat's outdoor territory and the cat's habit of drinking water from puddles *per se* were weak risk factors in the final multivariable case-matched analysis, but when these two variables were subjected to the interaction, the odds ratio (OR) increased to 5.3 indicating the potential risk factor for hyperthyroidism (Table 5). The following types of garden fertilisers were used in cat outdoor territory: blood and bone (21.9%), sheep pellets (18.5%), compost (17.8%), and a variety including combinations of

all of the mentioned above plus pig/chicken/cow/horse/llamas/fish/seaweed manure (41.8%). New Zealand with its well developed agricultural sector is a country where owners of private sections may use more natural fertilisers than artificial ones. However, the application of animal origin fertiliser could mirror the use of artificial fertilisers, pesticides, herbicides and insecticides on farms and/or could contain a factor which can trigger the changes within the thyroid gland itself.

Finally, the observation on drinking water from puddles could be explained in two ways. According to cat owners, 55% of the hyperthyroid cats show strong thirst (Table 8), therefore where there is a lack of availability of other sources of water, they are forced to drink e.g. from puddles, baths or containers with collected rain water. The last two variables, "water from bath" and "rain water", seem to be protective factors in univariate case-random analysis and in pre-final multivariable case-random analysis (Tables 9j and 10b in Appendices 1 and 2). Increased thirst, defined as the excessive daily intake of fluid reported by cat owners, was a potential risk factor for developing the hyperthyroidism in both pre-final multivariable analyses (Tables 10b and 11b, Appendices 2 and 3). This relationship could be false taking into account that polydipsia is a common sign (55%, Table 8) at the time of diagnosis of feline hyperthyroidism and here it is more an outcome of the disease than risk itself. However, the habit of drinking from puddles could point to an underlying problem with a cat's health, e.g. a mineral deficiency and it may be that the water from puddles provides that lacking mineral. On the other hand, the accuracy of observation on the cat drinking habits could point towards a more concerned, worried type of cat owner.

There is epidemiological and experimental evidence suggesting that environmental pollutants and certain medications may cause goitre by acting directly on the thyroid gland or indirectly by altering its regulatory mechanism and/or the peripheral metabolism and excretion of thyroid hormones. These pollutants operating in genetically predisposed individuals may also trigger pathogenic mechanisms in the thyroid gland much more easily than in non-genetically prone ones (Gaitan, 1988). Further research is needed to clarify these outdoor associations.

Diet

Daily canned food proportion and feeding a variety of canned food flavours

The present study, as was the case with two previous reports (Kass *et al.*, 1998; Scarlett *et al.*, 1988) identified increased odds ratios associated with an increasing proportion of canned food in the diet. Cats whose diets were less than ½ canned food had 1.6 times the risk of those fed no canned food at all, while cats fed a larger amount (≥ ½) of canned food in their diet had a 3.4 times greater risk of developing hyperthyroidism (Scarlett *et al.*, 1988). Study results of Kass *et al.* (1998) suggested a two- to three-fold increase in risk of developing hyperthyroidism among cats eating a diet composed mostly of commercial canned cat food.

This study demonstrated that eating half or more of daily food requirement as canned commercial cat food was associated with twice the risk of developing hyperthyroidism (final multivariable case-random model, see Table 4, page 73; Tables 9g and 10b, Appendices 1 and 2). As shown in Table 4, it was noticeable that, in spite of the fact that the p-values for both categories of canned food were not significant, the variable remained significant in the final model. Also, another interesting factor appeared in

Table 11a (Appendix 3) (pre-final multivariable case-matched analysis) in which up to ½ daily proportion of diet as a canned food acted as a protective factor. Additionally, the final case-matched model (Table 5) suggested that feeding a variety of canned food flavours could be a potential risk (OR=3.8) and this was complementary to the finding from case-random comparisons.

Furthermore, a diminished risk was found for cats which ate dry diets and with the increasing proportion of daily intake of it the protective value was increasing, Table 9g in Appendix 1. It is very difficult to interpret these findings, which are compatible with a harmful effect of commercial canned food but also with the protective effect of dry food. The relative proportions of canned and dry food were correlated. Not many cats consumed pet roll and/or pottle (plastic tub container) type of food, therefore no other substantial explanation was available.

Many cats ate other types of food such as fresh raw meat, cooked meat (dinner leftovers), dairy products and milk, cat treats or supplements. After a univariate case-random analysis on the above mentioned categories of foods, it was shown that:

(i) some of these diet ingredients were associated with higher risk for developing hyperthyroidism, e.g. eating raw meat; dairy products such as cheese, ice cream, cream, yoghurt, butter and milk (the last three dairy products are from multivariable case-matched analysis); cooked meat and eggs; adding medicines, vitamins and minerals including kelp and yeast to cat food (Tables 9g to 9i, Appendix 1; Table 10b, Appendix 2; Tables 11a and 11b, Appendix 3)

(ii) while other types of food were of protective nature, e.g. other sources of food such as small rodents and birds; feeding the cat vegetables/rice/fruits (the last one from pre-final multivariable case-

matched analysis; see Tables 9i, 10b and 11b in Appendices 1, 2 and 3 respectively).

In addition, in univariate case-random analysis it was found that cleaning the cat's food dish more than once a day and using a microwave oven to warm or defrost the cat's meals was associated with increased risk of developing hyperthyroidism (Table 9j Appendix 1).

A dietary factor has always been offered as a preferred hypothesis for the cause of hyperthyroidism in cats, mainly on account of the fact that there exists an array of thyroid disorders found in humans, which are caused by inadequate or excessive iodine ingestion, including goitre and hyperthyroidism.

There are some data in the cat canned food which suggest that commercial diets may play a role in developing hyperthyroidism. Canned foods may contain some ingredient(s) which, with prolonged exposure, either induce adenomatous hyperplasia or trigger clinical disease in a species that inherently has a high prevalence of this lesion. There is abundant evidence *in vivo* and *in vitro* for a role for iodine as a regulator of thyroid growth and function (Studer and Gerber, 1991).

The concentration of iodine varies widely in commercial cat food in the United States (Mumma *et al.*, 1986) and in New Zealand (Johnson *et al.*, 1992; Tarttelin and Ford, 1994), with some foods containing very small amounts and others, far above current recommendations for cats (up to 10 times) (AAFCO, 1993; Johnson *et al.*, 1992). Johnson *et al.* (1992) study of commercial cat food preparations showed that iodine concentration can vary more than 100-fold.

Excessive iodine ingestion can have a variety of physiological effects. The outcome depends on the functional state of the thyroid at the time of iodine ingestion. In general, a characteristic feature of iodine-induced hyperthyroidism in people is its transient course (Roti and Vagenakis, 1991), but once a hot nodule (toxic nodular goitre) is present, iodine supplements may cause permanent and progressive thyrotoxicosis. Administration of iodine can induce hyperthyroidism in human patients with non-toxic nodular goitre living in areas with sufficient dietary iodine; in healthy people living in endemic iodine-deficient areas (Roti and Vagenakis, 1991); and in euthyroid patients with autonomously functioning thyroid follicles (Blum *et al.*, 1976).

However, if excessive iodine ingestion was the sole cause of hyperthyroidism, it would not account for the continuous, autonomous and progressive nature of the disease in cats. Tarttelin *et al.* (1992) revealed that cats' thyroid hormone status is very sensitive to iodine intake, most likely explained by "Wolff-Chaikoff effect" (1948), a mechanism by which inorganic iodide uptake results in transient inhibition of organification of thyroid hormone and the reduced secretion of the formed hormone. The short-term (2 weeks) feeding of canned cat food of widely differing iodine content resulted in a dramatic thyroid response as measured by serum free T_4 (i.e. the higher iodide intake, the higher the urinary iodine excretion, the lower the T_4 concentration). That thyroid response (decreased T_4 concentration) may result in permanent thyroid disease, such as nodular goitre with or without hyperthyroidism.

Another study (Kyle *et al.*, 1994) showed that long-term (5 months) feeding of canned cat food varying in iodine content supported the concept that adaptive mechanisms tend to maintain the blood concentrations of thyroid hormone within the reference range in chronic states of high or low

dietary iodine intake. However, their data were insufficient to draw conclusions about what specific concentrations of intake are healthy or detrimental. Despite normal thyroid hormone homeostasis, it is well documented that in chronic states of iodine excess or deficiency, either state may finally lead to the development of goitre in humans and animals (Delange and Ermans, 1991; Marine, 1928; Nagataki, 1991; Roti and Vagenakis, 1991; Wollman and Breitman, 1970).

The univariate case-random analysis reported in this paper indicated an increased risk of developing hyperthyroidism when cats were regularly fed supplements such as vitamins and mineral tablets including kelp (high concentration of iodine), brewer's yeast along with Vegemite and Marmite, and various medicines (Table 9i, Appendix 1). Because these variables were characterised by a high number of missing observations, they were not subjected to multivariable analysis. Vegemite (Australian trademark) is a yeast extract, while Marmite (British trademark) is a yeast and vegetable derivative. Both are used as a spread or flavouring for stews.

Dogs and cats have a much greater daily fluctuation of serum T_4 values than humans due to lower hormone binding in serum and shorter serum half-lives (Kaptein *et al.*, 1994). In addition, thyroid hormones may be displaced from serum-binding proteins by endogenous or exogenous inhibitors, e.g. oleic acid, which is frequently added to commercial pet food.

In canned pet foods, there has been a trend towards lower ash content (mainly magnesium) and towards gourmet products containing more animal tissue. Iodine concentrations may be higher in gourmet cans containing marine fish or poultry products with remains of thyroid tissue

(Scarlett *et al.*, 1988). In the present study, there was no association between eating gourmet types of canned cat food and developing hyperthyroidism.

Also a xanthene dye, FD & C Red No. 3, is added frequently to pet food. This artificial dye contains iodinated compounds, which release free iodine during can thermal processing resulting in higher concentration of this element in the final product (Barbano and Dellavalle, 1984).

Another trace element, selenium, plays an important role in thyroid hormone metabolism as an essential component of the three deiodinases (Arthur *et al.*, 1996). Dietary selenium concentrations and the effects of its ingestion, and their relationship with feline hyperthyroidism have not been studied further. The selenium topic can become obscured by the widespread application of synthetic antioxidants use in commercial pet food manufacture (Dodds, 1995). The synthetic antioxidants have the potential, for example, to change the bioavailability of vitamin A (essential for many biochemical pathways including thyroid metabolism), vitamin E and selenium and may also alter cellular metabolism by inducing or lowering cytochrome P450, glutathione peroxidase (a selenium-dependent enzyme), and prostaglandin levels. The long term effects of synthetic preservatives and their potential indirect influence(s) on thyroid hormone metabolism should be investigated further.

Apart from iodine, most cat foods contain relatively high concentrations of goitrogenic compounds like phthalates, which can be found in fish products, milk and bovine tissue (Mayer *et al.*, 1972). There are many other goitrogenic substances (e.g. phenol derivatives, organochlorines with polychlorinated biphenyls [PCBs], polycyclic aromatic hydrocarbons [PAH],

azo dyes), which cats may be exposed to, either through their diets (particularly fish-based meals) or in the environment. Some of these could contribute to the development of thyroid adenomatous lesions found in hyperthyroid cats. Most of these substances are metabolised by glucuronidation, a process that is exceptionally slow in cats. The univariate case-random analysis in this study indicated increased risk of developing hyperthyroidism when cats ate fresh raw meat, cooked meat, eggs, dairy products and milk.

Some other dietary ingredients are goitrogenic too, e.g. calcium in rats on a low-iodine diet; contaminated drinking water; fluoride, rubidium, nitrate, and sulphur-containing compounds found in drinking water (McLaren and Alexander, 1979).

Also, some naturally occurring goitrogens such as soybean, some other beans, onion and garlic powder or oil, seaweed including kelp, gums from seaweed (carrageenan, alginates), and linseed are extensively used as ingredients in pet and human foods (e.g. pet rolls, ice cream, sweets).

Soybean products have been utilised very widely as an animal protein substitute in commercial pet food since 1933. These products are obtained by subjecting soybean to variety of technological processes. Only three of twenty soybean products are produced by a heating process. They were adopted in 1964 with amendment and second acceptance in 1971, in 1975 and in 1992 (AAFCO, 1993).

Although it seems unlikely that dietary goitrogens acting alone can cause goitre, it is quite possible that their effects may be additive when interacting with other factors, such as iodine deficiency or excess, including the day-to-

day wide swings of iodine content in the diets, or other types of goitrogen from the environment (from water, air and food) and may contribute to the overall increasing incidence of human and animal's goitre in endemic areas (Gaitan, 1988; McLaren and Alexander, 1979).

A consistent finding with all of these goitrogens mentioned earlier, by either physiologic perturbations or xenobiotic chemicals, is the hypersecretion of TSH, which by receptor-mediated events places the rodent thyroid gland at greater risk of developing benign or malignant tumours through a secondary mechanism of thyroid oncogenesis.

Glucuronidation should be mentioned here also. It is an important reaction for the metabolic clearance of a variety of endogenous and exogenous substances. The cats deficiency in glucuronide synthesis and the evidence of involvement of at least three UGT isoenzymes (UDP [uridine diphosphate]-glucuronyltransferases) in the glucuronidation of thyroid hormones in rats (Visser *et al.*, 1993) require a further investigation from the cats point of view. It is very likely that the constant exposure of cats to modern artificial goods/chemicals together with the cats slightly different genetic constitution for detoxification processes can act as a synergistic factor and as a result predispose cats to hyperthyroidism.

The combined effects of all of the above hypotheses need to be clarified in terms of the interrelationship between the different factors involved and their potential association with hyperthyroidism in cats.

History and clinical findings in New Zealand hyperthyroid cats

The clinical expression of the disease was not the primary interest of this study. However some data was obtained and it was compared with other studies for interest. The percentage of historical and clinical findings in affected cats was analysed and presented in Table 8. This study includes the percentage of affected cases reported separately by cat owners and by veterinarians. It is noticeable that the frequency of many history and clinical signs in the 1993 report (Broussard and Peterson, 1993; Broussard *et al*, 1995) was considerably decreased compared with the early 1980 studies (Hoenig *et al*., 1982; Holzworth *et al*., 1980; Peterson *et al*., 1983). Data from this present study follow the same trend with a few exceptions. Polyphagia, polypnoea and skin changes such as poor unkempt hair coat and alopecia had similar frequencies to those ones reported in early 1980 studies. While voice changes, decreased activity and aggressiveness/irritability were much more frequently found in New Zealand investigations, mainly when they were observed by cat owners, than in the five previous reports.

The severity of feline hyperthyroidism is thought to have reduced over the last 12 to 18 years since the first cases were reported, but clinical signs and physical examination findings still remain useful indicators of this disorder. The reduction of the clinical signs of hyperthyroid cats at the time of presentation to a veterinarian could reflect a higher awareness among veterinarians of that condition so that the cases do not have time to develop the full classical clinical picture.

Table 8. History and clinical findings in six independent series of cases of feline hyperthyroidism (NS=not specified; [a] includes powerful apex beat, gallop rhythm and arrhythmias; [b] includes tachypnoea, dyspnoea, coughing and sneezing)

Findings	Percentage of cases affected						
	This study (1999)		Broussard and Peterson (1993) Broussard *et al.* (1995)	Thoday and Mooney (1992)	Peterson *et al.* (1983)	Hoenig *et al.* (1982)	Holzworth *et al.* (1980)
	(125 cases)		(202 cases)	(126 cases)	(131 cases)	(24 cases)	(10 cases)
	owner	vet					
Weight loss	89	92	88	94	98	83	100
Polyphagia	74	68	49	78	81	79	70
Hyperactivity	31	34	31	56	76	38	70
Tachycardia		62	42	62	66	79	70
Polyuria/polydipsia	13/55	17/33	36	71	60	71	40-50
Vomiting	30	26	44	30	55	33	40
Heart murmur		27[a]	54	34[a]	53	NS	50
Diarrhoea	20	6	15	}51	33	54	50
Increased faecal volume	8	2	8		31	NS	50
Anorexia	NS	NS	7	NS	26	NS	NS
Polypnoea (panting)	22		9		25	33	NS
Heat intolerance	14	3	NS	NS	25	8	NS
Intermittent fever		2	NS	19	25	8	70
Muscle weakness	15	10	12	NS	25	NS	NS
Muscle tremor	8	1	15	NS	18	NS	NS
Congestive heart failure		1	2	3	12	NS	NS
Increased nail growth	12	12	6	NS	12	NS	NS
Dyspnoea		13[b]	10	38[b]	11	NS	NS
Skin changes:				}32	7	58	30
unkempt hair coat	48	45	9				
alopecia	6	4	3				
Ventral neck flexion		2	1	1	3	NS	NS
Hyperaemia of mucous membranes and skin		2	NS	NS	NS	NS	30
Voice change	36	6	NS	NS	NS	8	NS
Palpable thyroid gland		56	83	98	90	88	100
Intermittent decreased appetite	NS	NS	NS	2	NS	NS	NS
Decreased appetite	11	8	16	10	NS	NS	NS
Decreased activity	30	11	12	10	NS	NS	NS
Gallop rhythm			15		NS	NS	NS
Aggressiveness/irritability	33	24	10	NS	NS	NS	NS
Thin	NS	NS	65	NS	NS	NS	NS
Haematuria	NS	NS	NS	3	NS	NS	NS

Seasonality of feline hyperthyroidism diagnosis

The increased number of feline hyperthyroidism diagnoses in the warmer six months of the year, from October until March, could be accounted for in four different ways (Figure 18).

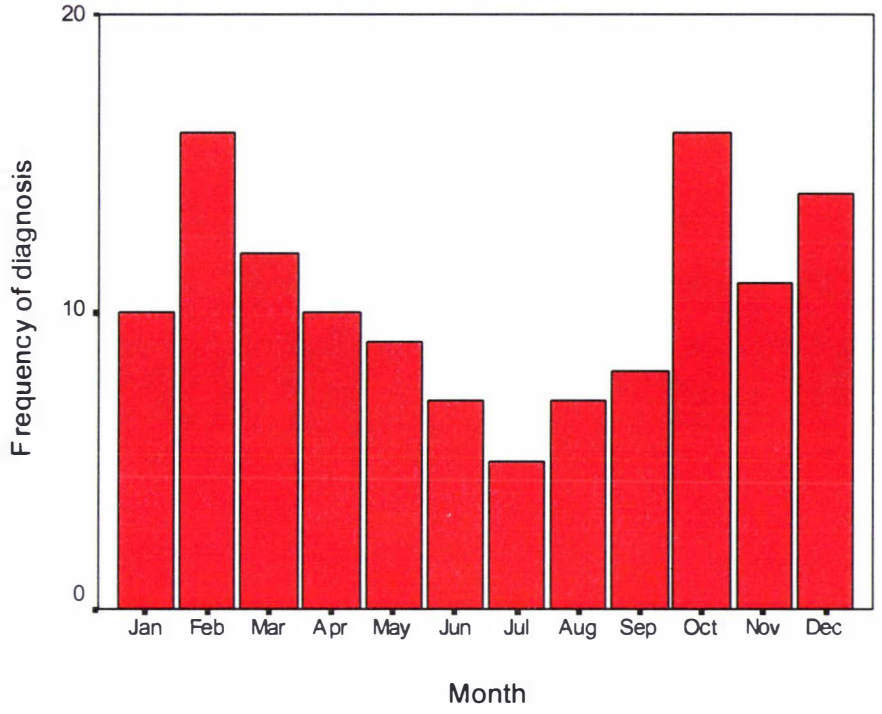


Figure 16. Pooled frequency of diagnosing hyperthyroid cats in New Zealand by month (125 cases)

Firstly, some of the clinical signs of hyperthyroidism, like hyperactivity, heat intolerance, intermittent increase of the body temperature or inability to control the body temperature, are augmented when the ambient temperature is higher. The seasonality of diagnosis of feline hyperthyroidism in New Zealand is exactly in agreement with the same periodicity of human thyrotoxicosis; as studied in Wellington, New Zealand by Ford in 1988. Ford suggested that patients were more likely to be diagnosed in the warmer weather as symptoms were less tolerable.

Secondly, those warmer months are connected with holiday time in New Zealand. The cats are often left at the catteries, where confirmation of vaccination for calicivirus, herpesvirus and panleukopenia is required. The cats are more likely to be presented to a veterinarian (and therefore more likely to be diagnosed with hyperthyroidism) for routine calicivirus, herpesvirus and panleukopenia vaccination and deworming at that time of the year. The drop in number of hyperthyroid diagnoses in January could be associated with the traditional holiday period in New Zealand and stricter financial budgeting after the high expenditure for Christmas in December. The high peak in February may to be associated with the coincidence of noticing clinical signs by cat owners after the animals are collected from the catteries.

The third explanation, could be linked to more time spent outdoors by cats during warmer months. Higher temperatures encourage cats to go outside. This fact increases the possibility of contacts with other cats (potential for developing cat fight/bite abscesses) and the probability for obtaining prey food. Most New Zealand domestic cats have a different lifestyle from US cats. This study revealed that New Zealand cats are predominantly outdoor cats. 72.3% of all cats participating in this study spent more than 3 hours a day outside; 71.5% of all cats slept predominantly inside the house at night and 94.1% of all cats had unrestricted access during 24 hours from the house to a relatively big outside area. From the univariate case-random comparison more than 3 hours per day spent outside was a protective factor (OR=0.5717) as well as occasional to frequent cat fights (OR=0.4856), in contrast to cat behaviour reported as lazy or very lazy which was associated with increased risk of developing hyperthyroidism (OR=2.7767) (Tables 9d and 9e, Appendix 1). These findings are in agreement with Scarlett *et al.*

(1988) 'cats living strictly indoors' identified as at increased risk for developing the hyperthyroidism.

From univariate case-random analysis, other sources of food, in particular prey food such as small rodents (mice) and birds and hunting behaviour were acting as protective factors with OR = 0.3432, OR = 0.4571, OR = 0.4809 and OR = 0.4641 respectively (Table 9i, Appendix 1). If an infectious agent is responsible for development of hyperthyroidism, it seems that it is not readily transmissible between cats and it is very unlikely that the rodents or birds are the potential natural reservoir of this agent. As mentioned earlier, prey food was acting as a protective factor with OR = 0.5 (Table 9i, Appendix 1).

The last potential explanation for the seasonality of feline hyperthyroidism in New Zealand could be linked to dietary iodine intake. From human studies, it is apparent that iodine intake has increased in Britain (Hall and Lazarus, 1987), Denmark (Haas *et al.*, 1988) and in New Zealand (Cooper *et al.*, 1984) and it has been suggested that dairy products contribute to dietary iodine intake. In this study it was found that eating dairy products and drinking milk were associated with an increased risk of feline hyperthyroidism. In New Zealand, probably, there is no seasonal variation in iodine content of milk because cows are pastured all year round, but iodophors are used as antiseptics in the dairy industry so that can influence the iodine content of milk. Milk iodine levels in New Zealand, in the period 1978 - 1979, when iodophor disinfectants were widely used in the dairy industry were from 0.1 to over 0.5 mg of iodine/kg of non - fat milk, while in the period 1987 - 1988, when other dairy disinfectants were preferred, they were less than 0.25 mg/kg (Sutcliffe, 1990). In general, concentrations of iodine in milks vary widely according to geographical region and intakes from the diet, dietary supplements, and iodine

containing pharmaceuticals (Casey *et al.*, 1995). Concentrations have been reported to be high in colostrum and decline with duration of lactation in cows and humans. New Zealand surveys found that iodine concentration in mature human milk is around 49 ng/ml (Johnson *et al.*, 1990), while for cow's milk 70 ng/ml (Sutcliffe, 1990) and 219 ng/ml (Johnson *et al.*, 1990). These figures are in agreement with the results reported from other countries, with the exception of a much higher figure reported by Johnson in 1990 (219 ng/ml). This high iodine concentration was observed for cow's milk in the Wellington area. Milk with that high iodine content would provide supraphysiological quantities of iodine when ingested. Additionally, iodine concentrations are low in lush, fast growing pasture in areas of high rainfall, which means that intake from pasture is likely to be the lowest in autumn and in late winter/early spring. Unfortunately, the present researcher was not able to find any more detailed data related to the above issues from a New Zealand perspective.

Conclusions

The present study has identified a number of risk factors that are statistically associated with the presence of feline hyperthyroidism. By the nature of the study, being an owner-directed, questionnaire-based, case-control study, it has been restricted to exploring some of the host and environmental epidemiological factors, and we can only propose biological explanations. Nevertheless, there are a number of themes that run through the study and support other published findings. These points are related to:

(i) age of the cat,
(ii) cat breed,
(iii) type of diet and feeding practices,
(iv) the pattern of use of insecticides (anti-flea treatment of cat's bed/bedding and frequent use of anti-fly sprays at home),
(v) regular application of fertilisers on cat's outdoor territory.

There was some evidence suggesting that alternative food sources such as catching and eating rodents and birds were protective, as were the calicivirus, herpesvirus and panleukopenia, and FeLV vaccinations. The vaccinations were directly connected with a routine veterinary check during the previous year. Some of those findings could be simply a marker for cats that receive 'better' (in human context) than average care, 'enjoy' longer lives, are very close to their owners (from the emotional perspective these owners are dependant on them), and are more likely to reach an age at which cats develop hyperthyroidism.

On the other hand, a strong support for breed predilection and a new finding of a gender predilection for the disease, suggest a strong genetic predisposition, but no work in this area has been carried out to-date.

Further thoughts on the aetiology of feline hyperthyroidism

The results of this observational study only provide useful leads to the aetiology of feline hyperthyroidism. However a case-control study aims to generate hypotheses – they then have to be tested by other research methods. More research is now necessary to:

- evaluate the role of cat breed on risk
- evaluate the role of cat sex and cat's age at desexing on chance of developing hyperthyroidism
- analyse the occurrence of "clusters" of hyperthyroid households including cats and humans
- characterise exposure of cats and humans to insecticides, along with anti-flea short and long acting formulations, insecticide and dye residues in wool used for carpet production, anti-fly preparations, anthelminthic residues and manure (animal and plant origin fertilisers), and explore the thyrotoxic potential of such exposures
- examine further the possible risk factors in indoor environments, e.g. different types of litter and bedding including sawdust, wood shavings and wool types
- research the owner - pet relationship to determine how it affects the environment from the holistic point of view
- evaluate the relative compositions and changes over time of canned versus dry commercial cat foods
- explore the frequency of multinodular adenomatous hyperplasia in cats in other countries taking into account feeding practices before and after the introduction of commercial canned and dry cat food
- investigate the current frequency of multinodular adenomatous goitre in cats without clinical signs during routine post mortem examinations

- explore thyroid tissue behaviour over the cats' life span together with the thyroid function in older, non hyperthyroid animals
- study the frequency of feline hyperthyroidism in other felid species kept in zoos, semi-captivity and in the wild
- explore the biochemical and molecular aspects of the glucuronide synthesis in healthy humans and humans diagnosed with toxic nodular goitre and compare these results with glucuronidation findings from healthy and hyperthyroid cats

As mentioned earlier, other studies have found similar associations between age, breed, diet, insecticide and fertiliser usage, and this study reinforces those findings. The possible effects of sex and, in particular, animal/plant origin (organic) fertilisers usage have not been reported before and warrant further investigations. In order to investigate these relationships further, one would need to resort to other epidemiological techniques, such as path analysis (Martin and Meek, 1986).

On the basis of the epidemiological findings made in this study, it is likely that feline hyperthyroidism may have a multifactorial aetiology. Genetic predisposition, environmental exposure to some factor(s) (e.g. cat litter, chemical sprays and fertilisers applied in cats territory), nutritional constituents (canned food), immunological factors, and even possibly infectious agent(s) may all influence the development of the disease. Further investigations may uncover the cause of this disease and may help in the development of adequate preventive measures, and eventually may lead to a lower percentage of affected cats. This would enable us to reduce the inconvenience of treating a hyperthyroid cat and reduce associated expenditure. The last two features would be warmly welcomed by cat owners.

When studying the epidemiological characteristics of a disease, it is important to conduct multiple studies, preferably in different populations and using different investigational strategies. Also, it is becoming increasingly clear that applications of concepts from clinical biochemistry and molecular genetics (e.g. representational difference analysis [RDA], Lisitsyn, 1995) can facilitate the identification of both risk factors for the individual cat and populations at risk for a number of diseases, including the ones with unknown aetiology. It is imperative now to start considering and applying molecular genetics and biochemical methods to support epidemiological studies of feline hyperthyroidism.

Bibliography

AAFCO - Association of American Feed Control Officials, 1993. Official Publication, Atlanta: AAFCO Inc., U.S.A.

Anon, SAS/STAT' Software: The Phreg Procedure Preliminary Documentation. In: SAS Institute Inc. Manual, pp. 37-41. North Carolina, U.S.A.

Anon, 1996. 1995 Companion animal study, University of Minnesota and Mark Morris Institute (editorial). Journal of Veterinary Dentistry 13: 9.

Anon, 1998. Operation clean fleece. Fly by jet. Wools of New Zealand, Wellington.

Anon, 1998a. Nonlinear Principal Components Analysis (PRINCALS). In: SPSS Categories® 8.0, pp. 27-33, SPSS Inc., Chicago, U.S.A.

Arthur, J.R., Bermano, G., Mitchell, J.H., Hesketh, J.E. 1996. Regulation of selenoprotein gene expression and thyroid hormone metabolism. Biochemical Society Transactions 24: 384-388.

Barbano, D.M., Dellavalle, M.E. 1984. Thermal degradation of FD & C Red No. 3 and release of free iodide. Journal of Food Protection 47: 668-669.

Barter, R.A., Klaassen, C.D. 1992. UDP-glucuronosyltransferase inducers reduce thyroid hormone levels in rats by an extrathyroidal mechanism. Toxicology and Applied Pharmacology 113: 36-42.

Barter, R.A., Klaassen, C.D. 1994. Reduction of thyroid hormone levels and alternation of thyroid function by four representative UDP-glucuronosyltransferase inducers in rats. Toxicology and Applied Pharmacology 128: 9-17.

Bidey, S.P. 1990. Control of thyroid cell and follicle growth: recent advances and current controversies. Trends in Endocrinology and Metabolism 1: 174-178.

Blaxter, A.C., Gruffydd-Jones, T.J. 1994. The Endocrine System. In: Feline Medicine and Therapeutics. Chandler, E.A. Gaskell, C.J., Gaskell, R.M. (ed.). 2nd edition, pp. 418-442. Oxford: Blackwell Scientific Publications.

Blum, M., Kranjac, T., Park, C.M., Engleman, R.M. 1976. Thyroid storm after cardiac angiography with iodinated contrast medium. Journal of the American Medical Association 235: 2324-2325.

Breslow, N.E., Day, N.E. 1980. Statistical Methods in Cancer Research, Vol. 1 – The analysis of case-control studies. IARC Scientific Publications No. 32. International Agency for Research on Cancer, Lyon, France.

Broussard, J.D., Peterson, M.E., Fox, P.R. 1995. Changes in the clinical and laboratory findings in cats with hyperthyroidism from 1983 - 1993. Journal of the American Veterinary Medical Association 206: 302-305.

Broussard, J.D., Peterson, M.E. 1993. Changes in the clinical and laboratory findings in hyperthyroid cats from 1982 - 1992. Abstract. In: Journal of Veterinary Internal Medicine 7: 112.

Brown, R.S., Keating, P., Livingston, P.G., et al. 1992. Thyroid growth immunoglobulins in feline hyperthyroidism. Thyroid 2: 125-130.

Brownlie, B.E.W., Wells, J.E. 1990. The epidemiology of thyrotoxicosis in New Zealand: incidence and geographical distribution in North Canterbury, 1983 - 1985. Clinical Endocrinology 33: 249-259.

Capen, C.C. 1994. Mechanism of chemical injury of thyroid gland. Progress in Clinical Biology Research 387:173-191.

Capen, C.C. 1997. Mechanistic data and risk assessment of selected toxic end points of the thyroid gland. Toxicologic Pathology 25: 39-48.

Casey, C. E., Smith, A., Zhang, P. 1995. Microminerals in human and animal milks. In: Handbook of Milk Composition. Jensen, R.G. (ed.), pp. 622-674. Academic Press, San Diego, U.S.A.

Clark, S.T., Meier,H. 1958. A clinico-pathological study of thyroid diseases in the dog and cat. Part 1. Thyroid pathology. Zentralblatt für Veterinarmedizin, Reihe A, 5: 17-32.

Cooper, G.J.S., Croxon, M.S., Ibbertson, H.K. 1984. Iodine intake in a urban environment: a study urine iodine excretion in Auckland. New Zealand Medical Journal 97:142-145.

Cotter, S.M. 1979. Uncommon disorders in the cat. In: Proceedings of the American Animal Hospital Association, 46th Annual Meeting, pp. 115-117.

Croxson, Michael 1997. Personal communication.

Delange, F., Ermans, A-M. 1991. Iodine deficiency. In: Werner and Ingbar's The Thyroid - A Fundamental and Clinical Text. Braverman, L.E., Utiger, R.D. (ed.), 6th edition, pp. 506-524. Philadelphia: J.B. Lippincott Company.

Dutton, G.J. (ed.) 1966. Glucuronic Acid: Free and Combined Chemistry, Biochemistry, Pharmacology and Medicine. Academic Press, New York, U.S.A.

Dodds, W.J. 1995. Overview: bridging basic science and clinical medicine. Advances in Veterinary Science and Comparative Medicine 39: 1-28.

Eckersall, P.D., McEwan, N.A., Mooney, C. 1991. An assessment of the CITE T4 immunoassay. Veterinary Record 129: 532-533.

EFS - Effem Foods Survey, 1997. Roy Morgan Omnibus Survey, Auckland, New Zealand.

Faber, E., Rubin, H. 1991. Cellular adaptation in the origin and development of cancer. Cancer Research 51: 2751-2761.

Ferguson, D.C. 1986. Treatments for hyperthyroidism. Feline Health Topics for Veterinarians 1: 1-6. Ithaca, New York: Cornell Feline Health Centre.

Ferguson, D.C. 1996. Nonthyroidal illness and drug effects on thyroid function tests: should we pay attention? In: Proceedings of the 14th American College of Veterinary Internal Medicine Forum, pp. 89-92. San Antonio, Texas, U.S.A.

Ford, H.C. 1988. Seasonality of thyrotoxicosis in Wellington. New Zealand Medical Journal 101: 72-73.

Florsheim, W.H., Velcoff, S.M., Williams, A.D. 1963. Some effects of 2,4-dichlorophenoxyacetic acid on thyroid function in the rat: effect on peripheral thyroxine. Endocrinology 72: 327-333.

Gaitan, E. 1988. Goitrogens. Baillière's Clinical Endocrinology and Metabolism 2: 683-702.

Gerber, H. 1993. Hyperthyroidism: pathogenesis of toxic nodular goiter in man and cat. In: Proceedings of the 11th American College of Veterinary Internal Medicine Forum, pp. 149-153.

Gerber, H., Peter, H.J., Bosinger, J., et al. 1991. Different continuous cell lines from adenomatous feline goitres widely differ in morphologic, functional and growth parameters. In: Progress in Thyroid Research. Gordon, A., Gross, J., Hennemann, G. (ed.), pp. 541-544. Rotterdam: Balkema.

Gerber, H., Peter, H., Ferguson, D.C., Peterson, M.E. 1994. Ethiopathology of feline toxic nodular goitre. Veterinary Clinics of North America: Small Animal Practice 24: 541-565.

Grebe, Stefan 1998. Personal communication.

Groch, K.M., Clifton, K.H. 1992. The plateau phase rat goitre contains a subpopulation of TSH responsive follicular cells capable of proliferation following transplantation. Acta Endocrinologica 126: 85-96.

Hall, R., Lazarus, J.H. 1987. Changing iodine intake and the effect on thyroid disease. British Medical Journal 294: 721-722.

Hass, V., Marley, M., Green, A., Date, J., Blichert-Toft, M., Mogensen, E.F. 1988. Urinary iodine excretion in a geographically stratified Danish population sample not affected by iodination programmes. Acta Endocrinologica (Copenhagen) 119: 125-131.

Hoenig, M., Goldschmidt, M.H., Ferguson, D.C., Koch, K., Eymontt, M.J. 1982. Toxic nodular goiter in the cat. The Journal of Small Animal Practice 23: 1-12.

Holzworth, J., Theran, P., Carpenter, J.L., Harpster, N.K., Todoroff, R.J. 1980. Hyperthyroidism in the cat: ten cases. Journal of the American Veterinary Medical Association 176: 345-353.

Hosmer, D.W. and Lemeshow, S. 1989. Applied Logistic Regression. John Wiley & Sons, New York, U.S.A.

Huber, G., Drewahl, M., Kaempf, J., et al. 1990. Generation of intercellular heterogeneity of growth and function in cloned rat thyroid cells (FRTL-5). Endocrinology 126: 1639-1645.

Johnson, L.A., Ford, H.C., Doran, J., Richardson, V.R. 1990. A survey of iodine concentration of human milk. New Zealand Medical Journal 103: 393-394.

Johnson, L.A., Ford, H.C., Tarttelin, M.F., Feek, C.M. 1992. Iodine content of commercially - prepared cat foods. New Zealand Veterinary Journal 40: 18-20.

Jones, B.R., Cayzer, J., Dillon, E.A., Smidt, K.P. 1991. Radio-iodine treatment of hyperthyroid cats. New Zealand Veterinary Journal 39: 71-74.

Jones, B.R., Hodge, H., Davies, E. 1995. The prevalence of feline immunodeficiency virus infection in hyperthyroid cats. New Zealand Veterinary Journal 43: 23-24.

Jones, B.R., Johnstone, A.C. 1981. Hyperthyroidism in an aged cat. New Zealand Veterinary Journal 29: 70-72.

Kaptein, E.M., Hays, M.T., Ferguson, D.C. 1994. Thyroid hormone metabolism. Veterinary Clinics of North America: Small Animal Practice 24: 431-463.

Kass, P. H., Peterson, M. E., Levy, J. K., James, K.M., Becker, D.V., Cowgill, L.D. 1998. Evaluation of environmental, nutritional, and host factors in cats

with hyperthyroidism. Abstract 64. In: Proceedings of 16th American College of Veterinary Internal Medicine Meeting, San Diego, California, U.S.A.

Kennedy, R.L., Thoday, K.L. 1984. Autoantibodies in feline hyperthyroidism. Veterinary Record 115: 575.

Kennedy, R.L., Thoday, K.L. 1988. Autoantibodies in feline hyperthyroidism. Research in Veterinary Science 45: 300-306.

Kleinbaum, D.G. 1996. Survival analysis: a self-learning text. Springer, New York, U.S.A.

Konijn, A.M., Gershon, B., Guggenheim, K. 1973. Further purification and method of action of a goitrogenic material from soybean flour. Journal of Nutrition 103: 378-383.

Kyle, A.H.M., Tarttelin, M.F., Cooke, R.R., Ford, H.C. 1994. Serum free thyroxine levels in cats maintained on diets relatively high or low in iodine. New Zealand Veterinary Journal 42: 101-103.

Labuc, R.H., Jones, B.R. 1986. Feline hyperthyroidism - a short review. Australian Veterinary Practitioner 16: 139-142.

Labuc, R.H., Jones, B.R. 1988. Feline hyperthyroidism - a review. New Zealand Veterinary Journal 36: 77-81.

Lisitsyn, N.A. 1995. Representational difference analysis: finding the differences between genomes. Trends in Genetics 11: 303-307.

Luo, G., Fan, J-L., Seetharamaiah, G.S, Desai, R.K., Dallas, J.S., Wagle, N., Doan, R., Niesel, D.W., Klimpel, G.R., Prabhakar, B.S. 1993. Immunisation of mice with *Yersinia enterocolitica* leads to the induction of antithyrotropin receptor antibodies. Journal of Immunology 151: 922-928.

Luo, G., Fan, J-L., Seetharamaiah, G.S., Niesel, D.W., Zhang, H., Peterson, J.W., Prabhakar, B.S., Klimpel, G.R. 1994. Purification and characterisation of *Yersinia enterocolitica* envelope proteins which induce

antibodies that react with human thyrotropin receptor. Journal of Immunology 152: 2555-2561.

Lucke, V.M. 1964. An histological study of thyroid abnormalities in the domestic cat. Journal of Small Animal Practice 5: 351-358.

Luttikhuis, L. 1989. Familial predisposition in feline hyperthyroidism. Canadian Veterinary Journal 30: 437.

Marine, D. 1928. Certain features of the morphologic pathology of endemic goitre. In: Comptes Rendus de la Conférence Internationale du Goitre. p.68. Bern: Hans Huber.

Martin, S.W., Meek, A.H. 1986. A path model of factors influencing morbidity and mortality in Ontario feedlot calves. Canadian Journal of Veterinary Research 50: 15-22.

Mayer, F.L. Junior, Stalling, D.L., Johnson, J.L. 1972. Phthalate esters as environmental contaminants. Nature 238: 411-413.

McKenzie, J.M., Zakarija, M. 1991. Antibodies in autoimmune thyroid disease. In: Werner and Ingbar's The Thyroid - A Fundamental and Clinical Text. Braverman, L.E., Utiger, R.D. (ed.), 6th edition, pp. 506-524. Philadelphia: J.B. Lippincott Company.

McLain, R.M. 1989. The significance of hepatic microsomal enzyme induction and altered thyroid function in rats: implications for thyroid gland neoplasia. Toxicologic Pathology 17: 294-306.

McLaren, E.H., Alexander, W.D. 1979. Goitrogens. Clinics in Endocrinology and Metabolism 8: 129-144.

Microsoft® Access 97, Relational Database Management System for Windows™ Version. Microsoft Corporation, U.S.A.

Middlesworth, L. van 1957. Thyroxine excretion, a possible cause of goitre. Endocrinology 61: 570-573.

Miyamoto, M., Sugawa, H., Mori, T., et al. 1988. Epidermal growth factor receptors on cultured neoplastic human thyroid cells and effects of

epidermal growth factor and thyrotropin on their growth. Cancer Research 48: 3652-3656.

Mooney, C.T., Little, C.J.L., Macrae, A.W. 1996. Effect of illness not associated with the thyroid gland on serum and free thyroxine concentrations in cats. Journal of the American Veterinary Medical Association 208: 2004-2008.

Mooney, C.T., Thoday, K.L., Doxey, D.L. 1996a. Serum thyroxine and triiodothyronine responses of hyperthyroid cats to thyrotropin. American Journal of Veterinary Research 57: 987-991.

Mumma, R.O., Rashid, K.A., Shane, B.S., Scarlett-Kranz, J.M., Hotchkiss, J.H., Eckerlin, R.H., Maylin, G.A., Lee, C.Y., Rutzke, M., Gutenmann, W.H., Bache, C.A., Lisk, D.J. 1986. Toxic and protective constituents in pet foods. American Journal of Veterinary Research 47: 1633-1637.

Nagataki, S. 1991. Autoregulation: effects of iodide. In: Werner and Ingbar's The Thyroid - A Fundamental and Clinical Text. Braverman, L.E., Utiger, R.D. (ed.), 6th edition, pp. 306-312. Philadelphia: J.B. Lippincott Company.

Nichols, David. 1998. Personal communication.

Nichols, R. 1996. New tests for the diagnosis of thyroid disease. In: Proceedings of the 14th American College of Veterinary Internal Medicine Forum, pp.87-88. San Antonio, Texas, U.S.A.

Nguyen L.Q., Jameson J.L., Stein B.S., Kopp P. 1998. Molecular cloning of the cat thyrotropin (TSH) receptor evidence against an autoimmune aetiology of feline hyperthyroidism. Abstract. In: Proceeding No. 3 of Genetic Conference, p.110, 26 June 1998. New Orleans, U.S.A.

Norušis, M.J. 1994. The logistic regression analysis. In: SPSS User's Guide SPSS Advanced Statistics™ 6.1, pp. 1-30. SPSS Inc., Chicago, U.S.A.

Norušis, M.J. 1994a. Kaplan-Meier survival analysis. In: SPSS User's Guide SPSS Advanced Statistics™ 6.1, pp. 275-290. SPSS Inc., Chicago, U.S.A.

Norušis, M.J. 1994b. Cox regression. In: SPSS User's Guide SPSS Advanced Statistics™ 6.1, pp. 291-328. SPSS Inc., Chicago, U.S.A.

Pearce, S.H., Foster, D.J., Imrie, H., Myerscough, N., Beckett, G.J., Thoday, K.L., Kendall-Taylor, P. 1997. Mutational analysis of the thyrotropin receptor gene in sporadic and familial feline thyrotoxicosis. Thyroid 7 (6): 923-927.

Peter, H.J., Gerber, H., Studer, H., Becker, D.V., Peterson, M.E. 1986. Morphology and function of goitres causing feline hyperthyroidism. Annual Endocrinology 47: 74.

Peterson, M.E. 1984. Feline hyperthyroidism. Veterinary Clinics of North America: Small Animal Practice 14: 809-826.

Peterson, Mark E. 1998. Personal communication.

Peterson, M.E., Gamble, D.A. 1990. Effect of nonthyroidal illness on serum thyroxine concentrations in cats: 494 cases (1988). Journal of the American Veterinary Medical Association 197: 1203-1208.

Peterson, M.E., Johnson, G.F., Andrews, L.K. 1979. Spontaneous hyperthyroidism in the cat. Abstract. In: 1st Proceedings of the American College of Veterinary Internal Medicine, p. 108. Seattle.

Peterson, M.E., Kintzer, P.P., Cavanagh, P.G., Fox, P.R., Ferguson, D.C., Johnson, G.F., Becker, D.V. 1983. Feline hyperthyroidism: pretreatment clinical and laboratory evaluation of 131 cases. Journal of the American Veterinary Medical Association 183: 103-110.

Peterson, M.E., Livingston, P., Brown, R.S. 1987. Lack of circulating thyroid stimulating immunoglobulins in cats with hyperthyroidism. Veterinary Immunology and Immunopathology 16: 277-282.

Peterson, M.E., Randolph, J.F., Mooney, C.T. 1994. Endocrine diseases. In: The Cat Diseases and Clinical Management. Sherding, R.G. (ed.), 2nd edition, volume 2, pp. 1403-1506. New York: Churchill Livingstone Inc.

Porter, W.P., Green, S.M., Debbink, N.L., et al. 1993. Groundwater pesticides: interactive effects of low concentration of carbamates aldicarb and methomyl and the triazine metribuzin on thyroxine and somatotropin levels in white rats. Journal of Toxicology and Environmental Health 40: 15-34.

Rothman, K.J. 1986. Modern Epidemiology. Little, Brown and Company, Boston/Toronto, U.S.A./Canada.

Roti, E., Vagenakis, A.G. 1991. Effects of excess iodide: clinical aspects. In: Werner and Ingbar's The Thyroid - A Fundamental and Clinical Text. Braverman, L.E., Utiger, R.D. (ed.), 6th edition, pp. 390-402. Philadelphia: J.B. Lippincott Company.

Roudebush, P. 1993. Pet food additives. Journal of the American Veterinary Medical Association 203: 1667-1670.

Scarlett, J.M. 1994. Epidemiology of thyroid diseases of dogs and cats. Veterinary Clinics of North America: Small Animal Practice 24: 477-486.

Scarlett, J.M., Moise, N.S., Rayl, J. 1988. Feline hyperthyroidism: a descriptive and case-control study. Preventive Veterinary Medicine 6: 295-309.

Schlesselman, J.J. 1982. Case-control studies: design, conduct, analysis. Oxford University Press, New York, U.S.A.

Singh, H., Singh, T.P. 1980. Thyroid activity and TSH potency of the pituitary gland and blood serum in response to Cythion and Hexadrin treatment in the freshwater catfish, *Heteropneustes fossils*. Environmental Research 22: 184-189.

Sparkes, A.H., Jones, B.R., Gruffydd-Jones, T.J., Walker, M.J. 1991. Thyroid function in the cat: assessment by the TRH response test and the thyrotrophin stimulation test. Journal of Small Animal Practice 32: 59-63.

SPSS ™, SPSS Inc., Chicago, U.S.A.

Stokes, M.E., Davis, C.S., Koch, G.G. 1995. Categorical Data Analysis Using the SAS System, pp. 267-275. Cary, North Carolina: SAS Institute Inc., U.S.A.

Studer, H., Gerber, H. 1991. Toxic multinodular goitre. In: Werner and Ingbar's The Thyroid - A Fundamental and Clinical Text, Braverman, L.E., Utiger, R.D. (ed.), 6th edition, pp. 692-697. Philadelphia: J.B. Lippincott Company.

Studer, H., Gerber, H. 1991a. Pathogenesis of nontoxic diffuse and nodular goitre. In: Werner and Ingbar's The Thyroid - A Fundamental and Clinical Text, Braverman, L.E., Utiger, R.D. (ed.), 6th edition, pp. 1107-1113. Philadelphia: J.B. Lippincott Company.

Studer, H., Peter, H.J., Gerber, H. 1985. Toxic nodular goitre. Clinics in Endocrinology and Metabolism 14: 351-372.

Studer, H., Peter, H.J., Gerber, H. 1989. Natural heterogeneity of thyroid cells: the basis for understanding thyroid function and nodular goitre growth. Endocrinology Review 10: 125-135.

Sutcliffe, E. 1990. Iodine in New Zealand milk. Food Technology in New Zealand 25:32-34 and 38.

Tarttelin, M.F., Ford, H.C. 1994. Dietary iodine level and thyroid function in the cat. American Institute of Nutrition. Journal of Nutrition 124: 2577S-2578S.

Tarttelin, M.F., Johnson, L.A., Cooke, R.R., Ford, H.C., Feek, C.M. 1992. Serum free thyroxine levels respond inversely to changes in levels of dietary iodine in the domestic cat. New Zealand Veterinary Journal 40: 66-68.

Tavris, D.R. 1997. Philosophy in Epidemiology and Public Health, p.7. Nova Science Publishers Inc., New York, U.S.A.

Taylor, J.A., Jacobs, R.M., Lumsden, J.H., Bonnett, B.N. 1989. Perspective on the diagnosis of feline hyperthyroidism. Canadian Veterinary Journal 30: 477-481.

Thoday, K.L. 1988. Feline hyperthyroidism - a review of the literature. Advances in Small Animal Practice 1: Proceedings of the British Small

Animal Veterinary Association, Chandler E.A. (ed.), pp. 120-158. Oxford: Blackwell Scientific Publications.

Thoday, K.L., Mooney, C.T. 1992. Historical, clinical and laboratory features of 126 hyperthyroid cats. Veterinary Record 131: 257-264.

Turrel, J.M. 1992. Feline hyperthyroidism - unravelling the diagnostic mystery. Proceedings of the 10th American College of Veterinary Internal Medicine Forum: 341-345.

Turrel, J.M., Feldman, E.C., Hays, M., Hornof, W.J. 1984. Radioactive iodine therapy in cats with hyperthyroidism. Journal of the American Veterinary Medical Association 184: 554-559.

Visser, T.J. 1994. Sulfation and glucuronidation pathways of thyroid hormone metabolism, pp85-117. In: Thyroid Hormone Metabolism. Molecular Biology and Alternate Pathways. Wu, S-y., Visser, T.J. (ed.), pp.85-117. CRC Press, Boca Raton, Florida, U.S.A.

Visser, T.J., van Haasteren, G.A., Linkels, E., Kaptein, E., van Toor, H., de Greef, W.J. 1996. Gender-specific changes in thyroid hormone-glucuronidating enzymes in rat liver during short-term fasting and long-term food restriction. European Journal of Endocrinology 135: 489-487.

Visser, T.J., Kaptein, E., van Toor, H., van Raaij, J.A., van den Berg, K.J., Joe, C.T., van Engelen, J.G., Brouwer, A. 1993. Glucuronidation of thyroid hormone in rat liver: effects of *in vivo* treatment with microsomal enzyme inducers and *in vitro* asssay conditions. Endocrinology 133: 2177-2186.

Volpé, R., 1991. Graves' disease. In: Werner and Ingbar's The Thyroid - A Fundamental and Clinical Text, Braverman, L.E., Utiger, R.D. (ed.), 6th edition, pp. 648-657. Philadelphia: J.B. Lippincott Company.

Wacholder, S., Silverman, D.T., McLaughlin, J.K., Mandel, J.S. 1992. Selection of controls in case-control studies III. Design options. American Journal of Epidemiology 135: 1042-1050.

Wase, A.W., Foster, W.C. 1956. Thiopental and thyroid metabolism. Proceedings of the Society for Experimental Biology and Medicine 91: 89-91.

Wilcke, J.R. 1984. Idiosyncracies of drug metabolism in cats. Effects on pharmacotherapeutics in feline practice. Veterinary Clinics of North America: Small Animal Practice 14: 1345-1354.

Wolff, J., Chaikoff, I.L. 1948. Plasma inorganic iodide as a homeostatic regulator of thyroid function. Journal of Biological Chemistry 174: 555-564.

Wollman, S.H., Breitman, T. R. 1970. Changes in DNA and weight of thyroid glands during hyperplasia and involution. Endocrinology 86: 322-327.

Appendix

Appendix 1

Tables 9a to 9j

Table 9a. Univariate analysis results showing significant variables ($p < 0.1$) and their categories for case and random control animals, displaying level of significance (p) for the variable, odds ratios (OR), 90% confidence intervals around the OR, and level of significance (p) for chi-squared test. Variables "*" were submitted to multivariable logistic regression analysis.

Cat and owner factors

Variables	p	OR (Exp(B))	90% CI for Exp(B)		Chi-Squared (model)	df	p
			Lower	Upper			
Moving house*	0.0057				11.208	2	0.0037
No		1.0					
Once	0.0050	2.7675	1.5233	5.0278			
Twice or more	0.2853	0.6648	0.3546	1.2463			
Length of living* at present address	0.0290				7.372	2	0.0251
Up to 3 years		1.0					
3 to 6 years	0.0330	2.2652	1.2056	4.2563			
> 6 years	0.0104	2.3048	1.3484	3.9395			
No. of children*	0.0446				8.722	3	0.0332
None		1.0					
One	0.7456	0.8777	0.4530	1.7006			
Two	0.0228	0.4260	0.2299	0.7892			
Three or more	0.0581	0.2737	0.0889	0.8427			
Females with thyroid diseases*					2.977	1	0.0845
No		1.0					
Yes	0.1365	5.1658	0.8420	31.6927			
Cat age*					157.773	2	0.0000
5 mths to 9 years	0.0000	1.0					
9 to 12 years	0.0000	13.3066	5.0613	34.9841			
> 12 years	0.0000	134.3967	51.6081	349.9932			
Length of cat ownership*	0.0000				104.516	4	0.0000
Up to 3 years		1.0					
3 to 6 years	0.0807	4.2550	1.0882	16.6375			
6 to 9 years	0.0247	6.5952	1.6575	26.2431			
9 to 12 years	0.0002	17.077	4.7753	61.0687			
> 12 years	0.0000	98.929	27.100	361.135			
Number of cat owners*					3.969	1	0.0463
One							
More than one (>6 months difference)	0.0489	1.8503	1.1067	3.0936			
Cat origin*					4.228	1	0.0398
"Unknown"		1.0					
"Known"	0.0407	0.5885	0.3843	0.9012			
Cat breed*	0.0287				18.274	3	0.0004
DSH		1.0					
DLH	0.5565	1.2161	0.7035	2.1022			
Siamese	0.0064	0.0585	0.0106	0.3243			
Other pure breeds	0.3175	2.3387	0.5779	9.4639			

Table 9b. Univariate analysis results showing significant variables ($p < 0.1$) and their categories for case and random control animals, displaying level of significance (p) for the variable, odds ratios (OR), 90% confidence intervals around the OR, and level of significance (p) for chi-squared test. Variables "*" were submitted to multivariable logistic regression analysis.

Cat factors including **medical history**

Variables	p	OR (Exp(B))	90% CI for Exp(B)		Chi-Squared (model)	df	p
			Lower	Upper			
Pure breeds	0.1032				7.732	2	0.0209
No (DSH/DLH)		1.0					
Bred in NZ	0.0362	0.3244	0.1340	0.7854			
Bred overseas	0.6889	0.0018	0.0000	316106394			
Cat sex*					7.797	1	0.0052
Male		1.0					
Female	0.0056	2.0393	1.3358	3.1134			
Cat age at desexing*	0.0047				11.374	2	0.0034
Up 6 or at 6 mths		1.0					
> 6 mths	0.0810	0.6123	0.3856	0.9723			
Don't know	0.0491	2.1359	1.1326	4.0281			
Calicivirus, herpesvirus & panleukopenia vaccine*					19.978	1	0.0000
No		1.0					
Yes	0.0000	0.2492	0.1454	0.4273			
Calicivirus, herpesvirus & panleukopenia vaccine frequency	0.0002				18.514	2	0.0001
Never given		1.0					
Given annually	0.0483	0.3887	0.1769	0.8541			
Given irregularly	0.4886	1.4480	0.6010	3.4883			
Feline leukaemia virus vaccine*					15.537	1	0.0001
No		1.0					
Yes	0.0066	0.0592	0.0107	0.3278			
Dental diseases*					20.242	1	0.0000
No		1.0					
Yes	0.0000	3.3629	2.1312	5.3065			
Respiratory tract diseases*					4.014	1	0.0451
No		1.0					
Yes	0.0487	1.9664	1.1184	3.4574			

Table 9c. Univariate analysis results showing significant variables ($p < 0.1$) and their categories for case and random control animals, displaying level of significance (p) for the variable, odds ratios (OR), 90% confidence intervals around the OR, and level of significance (p) for chi-squared test. Variables "*" were submitted to multivariable logistic regression analysis.

Cat medical history - continued

Variables	p	OR (Exp(B))	90% CI for Exp(B)		Chi-Squared (model)	df	p
			Lower	Upper			
Urinary tract diseases*					13.870	1	0.0002
No		1.0					
Yes	0.0004	3.4849	1.9488	6.2318			
Gastrointestinal tract diseases*					3.002	1	0.0832
No		1.0					
Yes	0.0918	2.1465	1.0189	4.5222			
Diarrhoea*					5.291	1	0.0214
No		1.0					
Yes	0.0242	2.1512	1.2299	3.7626			
Starvation*					9.159	1	0.0025
No		1.0					
Yes	0.0036	2.8398	1.5740	5.1237			

Table 9d. Univariate analysis results showing significant variables ($p < 0.1$) and their categories for case and random control animals, displaying level of significance (p) for the variable, odds ratios (OR), 90% confidence intervals around the OR, and level of significance (p) for chi-squared test. Variables "*" were submitted to multivariable logistic regression analysis.

Cat indoor and outdoor environment

Variables	p	OR (Exp(B))	90% CI for Exp(B)		Chi-Square (model)	df	p
			Lower	Upper			
Pasture*					2.885	1	0.0894
No		1.0					
Yes	0.0934	0.5589	0.3160	0.9887			
Industrial area*					4.065	1	0.0438
No		1.0					
Yes	0.0920	0.1600	0.0268	0.9574			
Time outside*					3.334	1	0.0679
0 to 3 h/day		1.0					
3 to 24 h/day	0.0705	0.5717	0.3438	0.9506			
Bedding-floor*					15.930	1	0.0001
No		1.0					
Yes	0.0001	3.4937	2.0355	5.9967			
Bedding-sheepskin*					2.865	1	0.0905
No		1.0					
Yes	0.0954	1.8355	1.0083	3.3412			
Bedding-wool*					11.632	1	0.0006
No		1.0					
Yes	0.0008	2.4255	1.5738	3.7382			
Cleaning method of bedding-wash*					2.829	1	0.0926
No		1.0					
Yes	0.0936	1.5468	1.0083	2.3730			
Sawdust used for bedding/litter trays*					3.291	1	0.0697
No		1.0					
Yes	0.0911	3.1480	1.0307	9.6146			
Other hyperthyroid cats in the household*					5.211	1	0.0224
No		1.0					
Yes	0.0639	7.3460	1.2510	43.1365			
No. of other hyperthyroid cats in the household	0.2983				5.824	2	0.0544
0		1.0					
One	0.1325	5.2542	0.8563	32.2416			
Two or more	0.6911	517.6415	0.0000	8.873E+13			

Table 9e. Univariate analysis results showing significant variables ($p < 0.1$) and their categories for case and random control animals, displaying level of significance (p) for the variable, odds ratios (OR), 90% confidence intervals around the OR, and level of significance (p) for chi-squared test. Variables "*" were submitted to multivariable logistic regression analysis.

Cat environment - continued

Variables	p	OR (Exp(B))	90% CI for Exp(B)		Chi-Square (model)	df	p
			Lower	Upper			
Hyperthyroid cat relation to cat under the survey					4.183	1	0.0408
No		1.0					
Yes	0.6279	504.7073	0.0000	7.512E+11			
Other pets in the household-rabbits					5.594	1	0.0180
No		1.0					
Yes	0.5752	0.0020	0.0000	172710.81			
Cat weight*	0.0000				64.829	2	0.0000
Normal	0.1204	1.0					
Heavier	0.0000	0.4879	0.2281	1.0434			
Lighter	0.0025	11.7091	6.1143	22.4234			
Cat behaviour*	0.0024				12.767	2	0.0017
Normal		1.0					
Active/energetic	0.8516	0.9360	0.5232	1.6745			
Lazy/very lazy	0.0010	2.7767	1.6671	4.6250			
Cat fighting behaviour*					7.901	1	0.0049
Never/seldom		1.0					
Occasionally/frequently	0.0053	0.4856	0.3171	0.7437			
Flea control at home*					7.767	1	0.0053
No		1.0					
Yes	0.0059	2.1627	1.3636	3.4302			
Flea control at home - products	0.0037				11.991	2	0.0025
None		1.0					
Short acting	0.3064	0.4276	0.1091	1.6762			
Long acting	0.0021	2.6236	1.5672	4.3922			
Flea control at home-frequency	0.0392		.		6.674	2	0.0355
0		1.0					
1 to 2 per year	0.0135	2.1527	1.2924	3.5858			
> 2 per year	0.4464	2.5597	0.3361	19.4971			

Table 9f. Univariate analysis results showing significant variables ($p < 0.1$) and their categories for case and random control animals, displaying level of significance (p) for the variable, odds ratios (OR), 90% confidence intervals around the OR, and level of significance (p) for chi-squared test. Variables "*" were submitted to multivariable logistic regression analysis.

Cat environment - continued

Variables	p	OR (Exp(B))	90% CI for Exp(B)		Chi-Square (model)	df	p
			Lower	Upper			
Chemical sprays on indoor plants*	0.1793				5.215	2	0.0737
None		1.0					
Pesticide/fungicide	0.0638	7.3553	1.2522	43.2048			
Pesticide	0.9598	1.0522	0.2003	5.5268			
Chemical sprays on indoor plants-frequency					3.292	1	0.0696
None		1.0					
1 to 15 x per year	0.0911	3.1494	1.0310	9.6204			
Chemicals added to flower vases					3.003	1	0.0831
No		1.0					
Yes	0.0874	0.5455	0.3044	0.9774			
Anti-flea products used regularly on cat's bed*					6.091	1	0.0136
No		1.0					
Yes	0.0328	5.3470	1.4693	19.4589			
Type of anti-flea products used regularly on cat*	0.0592				6.318	2	0.0425
None		1.0					
Short acting	0.9444	1.0196	0.6457	1.6100			
Long acting	0.0201	3.0272	1.3827	6.6277			
Use of mineral licks for farm animals*					3.291	1	0.0697
No		1.0					
Yes	0.0911	0.3177	0.1040	0.9702			

Table 9g. Univariate analysis results showing significant variables ($p < 0.1$) and their categories for case and random control animals, displaying level of significance (p) for the variable, odds ratios (OR), 90% confidence intervals around the OR, and level of significance (p) for chi-squared test. Variables "*" were submitted to multivariable logistic regression analysis.

Cat diet and feeding practices

Variables	p	OR (Exp(B))	90% CI for Exp(B)		Chi-Square (model)	df	p
			Lower	Upper			
Food alternation*					3.282	1	0.0700
No		1.0					
Yes	0.0750	1.9426	1.0518	3.5879			
Eating dry food					7.285	1	0.0070
No		1.0					
Yes	0.0111	0.3114	0.1463	0.6628			
Daily dry food proportion	0.0028				20.565	4	0.0004
None		1.0					
Up to 1/4	0.0624	0.4125	0.1888	0.9014			
> 1/4 to 1/2	0.0148	0.2988	0.1322	0.6753			
> 1/2 to 3/4	0.0044	0.0778	0.0178	0.3399			
> 3/4 to all	0.0013	0.0584	0.0137	0.2486			
Daily dry food proportion*	0.0006				19.281	2	0.0001
None		1.0					
Up to 1/2	0.0288	0.3644	0.1706	0.7787			
> 1/2 to all	0.0001	0.0667	0.0211	0.2110			
Daily can food proportion*	0.0018				13.169	2	0.0014
None		1.0					
Up to 1/2	0.8635	1.0875	0.4874	2.4265			
> 1/2 to all	0.0387	2.8546	1.2389	6.5777			
Eating raw meat*					8.356	1	0.0038
No		1.0					
Yes	0.0044	2.2335	1.4043	3.5523			
Frequency of raw meat feeding	0.0179				8.282	2	0.0159
None		1.0					
1x 2 weeks or less	0.0412	2.5454	1.1988	5.4044			
daily to 1x week	0.0081	2.1538	1.3371	3.4694			
Beef meat/mince/ fat-raw					4.357	1	0.0369
No		1.0					
Yes	0.0378	1.7174	1.1190	2.6356			
Beef offal-raw					7.600	1	0.0058
No		1.0					
Yes	0.0068	2.2992	1.3857	3.8150			
Fish meat-raw					6.739	1	0.0094
No		1.0					
Yes	0.0116	2.4457	1.3655	4.3804			

Table 9h. Univariate analysis results showing significant variables ($p < 0.1$) and their categories for case and random control animals, displaying level of significance (p) for the variable, odds ratios (OR), 90% confidence intervals around the OR, and level of significance (p) for chi-squared test. Variables "*" were submitted to multivariable logistic regression analysis.

Cat diet - continued

Variables	p	OR (Exp(B))	90% CI for Exp(B)		Chi-Square (model)	df	p
			Lower	Upper			
Dairy products*					8.848	1	0.0029
No		1.0					
Yes	0.0032	2.1701	1.4076	3.3458			
Frequency of dairy products feeding	0.0038				12.133	2	0.0023
None		1.0					
1x 2 weeks or less	0.0016	3.7911	1.8921	7.5990			
daily to 1x week	0.0788	1.6836	1.0339	2.7415			
Eating cheese					9.913	1	0.0016
No		1.0					
Yes	0.0021	2.4943	1.5312	4.0632			
Eating ice cream					4.739	1	0.0295
No		1.0					
Yes	0.0373	2.6519	1.2274	5.7297			
Eating cream					12.799	1	0.0003
No		1.0					
Yes	0.5515	1445.831	0.0000	7.749E+11			
Eating cooked meat*					7.097	1	0.0077
No		1.0					
Yes	0.0082	1.9722	1.2928	3.0088			
Frequency of cooked meat feeding	0.0293				7.170	2	0.0277
None		1.0					
1x 2 weeks or less	0.0253	2.0833	1.2146	3.5735			
daily to 1x week	0.0386	1.8849	1.1387	3.1201			
Cooked beef, sheep, pork meat					11.696	1	0.0006
No		1.0					
Yes	0.0009	2.8223	1.6868	4.7221			
Cooked chicken meat					2.778	1	0.0955
No		1.0					
Yes	0.0993	1.7503	1.0012	3.0598			
Cooked fish meat					4.618	1	0.0316
No		1.0					
Yes	0.0334	1.8585	1.1510	3.0009			
Eating eggs					5.548	1	0.0185
No		1.0					
Yes	0.0251	2.8333	1.3188	6.0872			

Table 9i. Univariate analysis results showing significant variables ($p < 0.1$) and their categories for case and random control animals, displaying level of significance (p) for the variable, odds ratios (OR), 90% confidence intervals around the OR, and level of significance (p) for chi-squared test. Variables "*" were submitted to multivariable logistic regression analysis.

Cat diet - continued

Variables	p	OR (Exp(B))	90% CI for Exp(B)		Chi-Square (model)	df	p
			Lower	Upper			
Adding supplements /medicines to cat food*					10.408	1	0.0013
No		1.0					
Yes	0.0023	3.3196	1.7382	6.3397			
Frequency of adding supplements /medicines	0.0059				11.826	2	0.0027
None		1.0					
1x 2 weeks or less	0.1671	4.7421	0.7435	30.2472			
daily to 1x week	0.0032	3.5567	1.7508	7.2253			
Adding vitamins/minerals					4.394	1	0.0361
No		1.0					
Yes	0.0434	2.4595	1.1821	5.1174			
Adding yeast					3.291	1	0.0697
No		1.0					
Yes	0.0911	3.1480	1.0307	9.6146			
Adding medicines					9.895	1	0.0017
No		1.0					
Yes	0.6003	1421.326	0.0000	1.123E+13			
Other source(s) of food*					16.899	1	0.0000
No		1.0					
Yes	0.0001	0.3432	0.2219	0.5308			
Prey-mice					8.690	1	0.0032
No		1.0					
Yes	0.0036	0.4571	0.2939	0.7111			
Prey-birds					8.181	1	0.0042
No		1.0					
Yes	0.0046	0.4809	0.3145	0.7352			
Hunting					5.499	1	0.0190
No		1.0					
Yes	0.0198	0.4641	0.2699	0.7979			
Daily quantity of fluid drunk*	0.0005				27.502	2	0.0000
Minimal		1.0					
Normal	0.1641	0.5851	0.3105	1.1025			
Excessive	0.0054	9.7210	2.5371	37.2470			

Table 9j. Univariate analysis results showing significant variables ($p < 0.1$) and their categories for case and random control animals, displaying level of significance (p) for the variable, odds ratios (OR), 90% confidence intervals around the OR, and level of significance (p) for chi-squared test. Variables "*" were submitted to multivariable logistic regression analysis.

Cat diet - continued

Variables	p	OR (Exp(B))	90% CI for Exp(B)		Chi-Square (model)	df	p
			Lower	Upper			
Drinks rain water*					3.433	1	0.0639
No		1.0					
Yes	0.0651	0.6161	0.4000	0.9488			
Drinks water from the bath*					4.980	1	0.0256
No		1.0					
Yes	0.0295	0.4501	0.2462	0.8228			
Time dry food stay in the dish	0.0065				11.001	2	0.0041
Not applicable		1.0					
Up 15 min	0.0021	0.2160	0.0951	0.4904			
> 15 min	0.0364	0.3746	0.1732	0.8105			
Time moist food stay in the dish	0.7553				6.726	2	0.0346
Not applicable		1.0					
Up 15 min	0.5789	479.4585	0.0000	4.217E+10			
> 15 min	0.5709	546.1372	0.0000	4.809E+10			
Dish cleaning frequency*					5.686	1	0.0171
Once a day or less		1.0					
2 to 5 x a day	0.0187	2.0289	1.2367	3.3287			
Use of microwave oven*					5.298	1	0.0213
No		1.0					
Yes	0.0234	2.0416	1.2161	3.4275			
Frequency of microwave use	0.0596				5.968	2	0.0506
None		1.0					
Occasionally	0.2130	1.6528	0.8511	3.2095			
1 to 2 x a day to 1 x a week	0.0315	2.6242	1.2548	5.4877			

Appendix 2

Tables 10a to 10b

Table 10a. Results of pre-final forward stepwise multiple unconditional logistic regression analysis of all significant variables ("*" from univariate analysis) for case and random control animals, showing level of significance (p) for the variable, odds ratios (OR) and 95% confidence intervals around the OR. Variable "#" was excluded from the final multivariable forward stepwise unconditional logistic regression analysis.

Variables	p	OR (Exp(B))	95% CI for Exp(B)	
			Lower	Upper
Cat age	0.0000			
5 mths to 9 years		1.0		
9 to 12 years	0.0000	14.5502	4.2027	50.3742
> 12 years	0.0000	229.2636	61.4969	854.7068
Cat breed	0.0449			
DSH		1.0		
DLH	0.5726	1.3383	0.4864	3.6819
Siamese	0.0080	0.0392	0.0036	0.4299
Other pure breeds	0.4520	3.7803	0.1182	120.9375
Cat sex				
Male				
Female	0.0034	3.7910	1.5546	9.2450
Cat age at desexing	0.0083			
Up 6 or at 6 months		1.0		
> 6 months	0.1287	0.4842	0.1900	1.2342
Don't know	0.0615	3.2680	0.9445	11.3070
Calici, herpesvirus & panleukopenia vaccine				
No		1.0		
Yes	0.0271	0.3977	0.1756	0.9008
Feline leukaemia virus (FeLV) vaccine				
No		1.0		
Yes	0.0021	0.0332	0.0038	0.2918
Dental diseases				
No		1.0		
Yes	0.0010	2.7861	1.5139	5.1273
Respiratory tract diseases				
No		1.0		
Yes	0.0474	2.1707	1.0091	4.6694
Urinary tract diseases				
No		1.0		
Yes	0.0006	4.2996	1.8698	9.8871
Starvation (abstinence from food)				
No		1.0		
Yes	0.0362	2.4708	1.0601	5.7586
Pasture				
No		1.0		
Yes	0.0183	0.3241	0.1271	0.8261
Bedding-floor				
No		1.0		
Yes	0.0001	5.6589	2.3498	13.6280

Table 10b. Results of pre-final forward stepwise multiple unconditional logistic regression analysis of all significant variables ("*" from univariate analysis) for case and random control animals, showing level of significance (p) for the variable, odds ratios (OR) and 95% confidence intervals around the OR. Variable "#" was excluded from the final multivariable forward stepwise unconditional logistic regression analysis.

Variables	p	OR (Exp(B))	95% CI for Exp(B)	
			Lower	Upper
Cat weight #	0.0000			
Normal		1.0		
Heavier	0.0000	0.1972	0.0621	0.6257
Lighter	0.0000	12.7345	5.0465	32.1344
Cat behaviour	0.0355			
Normal		1.0		
Active/energetic	0.5179	0.7286	0.2791	1.9023
Lazy/very lazy	0.0211	2.6449	1.1569	6.0471
Cat fighting behaviour				
Never/seldom		1.0		
Occasionally/frequently	0.0011	0.3007	0.1464	0.6177
Anti-flea products used regularly on cat's bed				
No		1.0		
Yes	0.0102	32.5026	2.2865	462.0260
Daily can food proportion	0.0016			
None		1.0		
Up to 1/2	0.8608	1.1082	0.3515	3.4938
> 1/2 to all	0.0379	3.5602	1.0737	11.8048
Eating raw meat				
No		1.0		
Yes	0.0003	3.6456	1.8080	7.3511
Other source(s) of food-prey (mice, birds)				
No		1.0		
Yes	0.0001	0.2937	0.1587	0.5434
Daily quantity of fluid drunk	0.0004			
Minimal		1.0		
Normal	0.4044	0.6815	0.2767	1.6786
Excessive	0.0021	15. 3524	2.6952	87.4493
Drinking rain water				
No		1.0		
Yes	0.0435	0.5269	0.2829	0.9816

Cat and owner factors: Residual Chi Squared = 6.984 with 10 df Sig = 0.7270
Cat medical factors: Residual Chi Squared = 3.357 with 6 df Sig = 0.7628
Cat environment factors: Residual Chi Squared = 15.742 with 12 df Sig = 0.2033
Cat diet factors: Residual Chi Squared = 13.572 with 9 df Sig = 0.1384

Appendix 3

Tables 11a to 11b

Table 11a. Pre-final forward stepwise multiple conditional logistic regression model for case and matched control animals, showing level of significance (p) for the variable, odds ratios (OR) and 95% confidence intervals around the OR. Variable "#" was excluded from the final multivariable forward stepwise conditional logistic regression analysis.

Variables	p	OR (Exp(B))	95% CI for Exp(B)	
			Lower	Upper
Cat breed	0.4558			
DSH		1.0		
DLH	0.6910	0.8604	0.4099	1.8059
Siamese	0.9496	1.953E-06	2.004-183	1.904+171
Other pure breeds	0.1083	0.3655	0.1070	1.2485
Cat age at desexing	0.0166			
Up 6 or at 6 months		1.0		
> 6 months	0.5364	1.2315	0.6365	2.3827
Don't know	0.0044	3.4535	1.4712	8.1064
Cat fight/bite abscesses				
No		1.0		
Yes	0.0460	1.7757	1.0103	3.1210
Diarrhoea				
No		1.0		
Yes	0.0178	2.7079	1.1876	6.1741
No. of other cats in house	0.0142			
0		1.0		
One	0.0081	0.2898	0.1158	0.7249
Two or more	0.0180	0.2205	0.0630	0.7718
Cat weight #	0.0000			
Normal		1.0		
Heavier	0.0693	0.3033	0.0837	1.0990
Lighter	0.0000	13.7521	4.2278	44.7329
Regular use of manure on cat's outdoor territory				
No		1.0		
Yes	0.0016	5.1036	1.8529	14.0571
Regular use of fly sprays on cat's indoor territory				
No		1.0		
Yes	0.0038	7.3458	1.9069	28.2986
Daily canned food proportion	0.0166			
None		1.0		
Up to 1/2	0.0324	0.0960	0.0112	0.8217
> 1/2 to all	0.2760	0.3401	0.0489	2.3674
Feeding a variety of can food flavours				
No		1.0		
Yes	0.0011	21.6218	3.3905	137.8856
Feeding cat dairy products				
No		1.0		
Yes	0.0082	2.6945	1.2922	5.6185

Table 11b. Pre-final forward stepwise multiple conditional logistic regression model for case and matched control animals, showing level of significance (p) for the variable, odds ratios (OR) and 95% confidence intervals around the OR. Variable "#" was excluded from the final multivariable forward stepwise conditional logistic regression analysis.

Variables	p	OR (Exp(B))	95% CI for Exp(B)	
			Lower	Upper
Feeding cat vegetables/rice/fruits				
No		1.0		
Yes	0.0060	0.1908	0.0585	0.6216
Daily quantity of fluid drunk	0.0032			
Minimal		1.0		
Normal	0.1112	2.3951	0.8176	7.0166
Excessive	0.0009	14.3410	2.9728	69.1824
Drinks water from puddles				
No		1.0		
Yes	0.0352	2.1076	1.0530	4.2182
Drinks milk				
No		1.0		
Yes	0.0011	3.9901	1.7341	9.1811

Cat and owner factors:	Residual Chi Squared = 26.75 with 23 df Sig = 0.2668
Cat medical factors:	Residual Chi Squared = 12.49 with 22 df Sig = 0.9464
Cat environment factors:	Residual Chi Squared = 83.19 with 76 df Sig = 0.2678
Cat diet factors:	Residual Chi Squared = 37.25 with 42 df Sig = 0.6794

Appendix 4

Table 12

Table 12. List of the rest of the variables and their categories which were found significant ($p < 0.1$) when subjected to univariate analysis for case-random control animals. These variables were not included in the multivariate forward stepwise logistic regression analysis because of a very high number of missing data points or a very low number of observations. First category of all variables was used as a reference category. (GIT = gastrointestinal tract).

Variable	Variable categories	p-value
Cat and owner factors		
Death of surveyed cat	No; Yes	0.0027
Cat medical history		
Chlamydia vaccine	No; Yes	0.0717
Number of visits to the veterinarian over the last 3 years - how many times it was for vaccination	None; Twice; Three times	0.0008 0.0000
Other medical conditions - type	None; GIT; Skin; Other	0.0937 0.5246 0.2862
Feline leukaemia virus (FeLV) test results	Not tested; Positive; Negative	0.7037 0.0610
Feline immunodeficiency virus (FIV) test results	Not tested; Positive; Negative	0.7032 0.0757
Cat indoor and outdoor environment		
Indoor toilet - litter type - sawdust	No; Yes	0.0905
Cat diet and feeding practices		
Any extra food eaten while on medical diet	No; Yes	0.0486
Brand of dry food - Hill's Science diets	No; Yes	0.0713
Flavours of dry food - chicken	No; Yes	0.0193
Flavours of dry food - calcium/bone	No; Yes	0.0707
Brands of canned food - canned fish for cats	No; Yes	0.0725
Brands of canned food - Kite Kat	No; Yes	0.0910
Flavour of canned food - beef offal - liver, kidney, heart	No; Yes	0.0563
Flavour of canned food - sheep meat - lamb/mutton	No; Yes	0.0065
Flavour of canned food - fish (ocean [tuna, pilchards, mackerel] & ocean/freshwater [salmon], and seafood [prawn])	No; Yes	0.0987
Flavour of canned food - game - venison	No; Yes	0.0929
Raw fresh meat storage before opening - how long (days) (fridge/freezer)	0-3 days; 4-180 days	0.0122
Type of serving dish	None; Glass; Other	0.0799 0.1910
Cleaning of serving dish - brand name of dishwashing liquid	No; Any; Sunlight	0.3113 0.0672

Appendix 5

Tables 13a to 13g

Table 13a. List of all variables which were found not to be significant when subjected to univariate analyses from cases, random and matched controls cats. All other variables are shown in Tables 9a to 9j and Table 12. First category of all variables was used as a reference category. (GIT = gastrointestinal tract).

Variable	Variable categories
Cat and owner factors	
Residence type	City; Town; Rural
Number of humans-females in the household	None; One; Two; Three or more
Number of humans-males in the household	None; One; Two; Three or more
Humans with any thyroid disorders	No; Yes
Humans with any thyroid disorders	Hyperthyroidism; Hypothyroidism
Males with thyroid disorders	No; Yes
Length of cat's hair	Short; Long
Cat medical history	
No of visits to veterinarian over the last 3 years	0; 1-2; 3-8; >8
Frequency of worming	Never; Annually; Irregularly
Origin of anti-worm preparations	Supermarket; Veterinary clinic
Allergy - skin	No; Yes
Allergy - food	No; Yes
Poisoning (toxicity)	No; Yes
Cancer	No; Yes
Other medical conditions	No; Yes
Other medical conditions - type	No; GIT; Skin; Other
Comments on cat health/behaviour	No; GIT; Behaviour;Other;Healthy
Tested for FeLV	No; Yes
Tested for FIV	No; Yes
Cat indoor and outdoor environment	
Size of cat's territory	400-1500m^2; 90-200m^2; >1500m^2
Lawn in cat territory	No; Yes
Garden in cat territory	No; Yes
Orchard in cat territory	No; Yes
Crop fields in cat territory	No; Yes
Park in cat territory	No; Yes
Play ground fields in cat territory	No; Yes
Other areas in cat territory	No; Indoor type; Outdoor type
Any time easy access to outside	No; Yes
Sleeping place at night	Inside; Outside; Other; Inside & outside
Type of bedding - human bed	No; Yes
Type of bedding - sofa/couch	No; Yes
Type of bedding - armchair/chair	No; Yes
Type of bedding - bean bag	No; Yes
Type of bedding - cane or plastic basket/dry bed	No; Yes
Type of bedding - cardboard box	No; Yes
Type of bedding – cotton and cotton/polyester mix	No; Yes
Type of bedding - acrylic & vinyl (furniture/tile)	No; Yes
Type of bedding - wood - furniture/floor/box/ kennel/ window sill	No; Yes
Type of bedding - newspapers/paper	No; Yes
Type of bedding - leaves/dirt/soil	No; Yes

Table 13b. List of all variables which were found not to be significant when subjected to univariate analyses from cases, random and matched controls cats. All other variables are shown in Tables 9a to 9j and Table 12. First category of all variables was used as a reference category. (GIT = gastrointestinal tract).

Variable	Variable categories
Cat environment - continued	
Type of bedding - feather duvet/cushion	No; Yes
Type of bedding - saw dust/wood shavings	No; Yes
Bedding cleaning	No; Yes
Bedding cleaning method - other than washing	No; Yes
Bedding cleaning frequency/year	1-36 times; >52 times
Indoor toilet - litter tray	No; Yes
Indoor toilet - litter type - dirt/soil/sand/potting mix	No; Yes
Indoor toilet - litter type - saw dust	No; Yes
Indoor toilet - litter type - commercial litter	No; Yes
Origin of sawdust/wood shavings used for bedding/litter	None; Mill; Shop
Type of sawdust/wood shavings used for bedding/litter	None; Untreated; Treated
Other pets at home - dog	No; Yes
Other pets at home - aviary birds	No; Yes
Other pets at home - Bantam hens/pigeons/ostriches	No; Yes
Other pets at home - fish/turtle/tortoise	No; Yes
Other pets at home - guinea pigs/rats/mice	No; Yes
Other pets at home - horse/donkey/pony	No; Yes
Cat ever left in a boarding cattery	No; Yes
Boarding cattery - how many times per year	0; 1-2 x in life; 1/year; >1/year
Boarding cattery - length of stay	0; up 7 days; 8-14 days; >14 days
Cat show	No; Yes
Cat shows - how many times a year	1; up 3/year; 4-5/year
Name/type of manure (animal/plant origin [organic] fertilisers)	None; Variety; Compost; Sheep pellets; Blood & bone
Frequency of manure application/year	0; 1-2/year; >2/year
Application of artificial fertilisers on cat territory	No; Yes
Name of artificial fertiliser product(s)	None; Variety; NPK based
Frequency of artificial fertilisers application/year	0; 1-2/year; >2/year
Application of herbicides on cat territory	No; Yes
Name of herbicide product(s)	None; Variety; Roundup
Frequency of herbicides application/year	0; 1-2/year; >2/year
Application of pesticides on cat territory	No; Yes
Name of pesticide product(s)	None; Pesticides - variety ; Pesticide & fungicide
Frequency of pesticides application/year	0; 1-2/year; >2/year
Application of fungicides on cat territory	No; Yes
Name of fungicide product(s)	None; Fungicide - variety; Fungicide & pesticide
Frequency of fungicides application/year	0; 1-2/year; >2/year
Application of snail & slug baits on cat territory	No; Yes
Name of snail & slug bait product(s)	None; Variety; Blitzem
Frequency of snail & slug baits application/year	0; 1-2/year; >2/year
Application of pest control poisons on cat territory	No; Yes
Name of pest control poison product(s)	None; Variety;

Table 13c. List of all variables which were found not to be significant when subjected to univariate analyses from cases, random and matched controls cats. All other variables are shown in Tables 9a to 9j and Table 12. First category of all variables was used as a reference category. (GIT = gastrointestinal tract).

Variable	Variable categories
Cat environment - continued	
Frequency of pest control poisons application/year	0; 1-2/year; >2/year
Application of other chemical substance(s) on cat territory	No; Yes
Name of pest control poisons product(s)	None; Rat/mice/ant killers
Frequency of pest control poisons application/year	0; 1-2/year; >2/year
Application of other chemical substance(s) on cat territory	No; Yes
Name of other chemical substance(s)	None;Fruit/veges/tree sprays;Other
Frequency of other chemical substance application/year	0; 1-2/year; >2/year
Name of fly spray product(s)	None; Raid; Black Flag;Other
Frequency of fly spray application/year	0; 1-12/year; >12/year
Application of ant killers indoors	No; Yes
Name of ant killers product(s)	None; Boric acid/borax; Pyrethrins
Frequency of ant killers application/year	0; 1-12/year; >12/year
Using indoor plant fertiliser(s)	No; Yes
Name of indoor plant fertiliser(s)	None; Variety; NPK based
Frequency of using indoor plant fertilisers/year	0; 1-6/year; >6/year
Application of any chemical sprays on indoor plants	No; Yes
Application of baits for pests indoors	No; Yes
Name of bait(s) for pests used indoors	None; Rat/mice/ant killers
Frequency of using indoor baits for pests/year	0; 1-6/year; >6/year
Application of other chemical substance(s) indoors	No; Yes
Name of other chemical substance(s) used indoors	None; Household; Commercial
Frequency of other chemical substance(s) indoor application/year	0; 1/year; >1/year
Cat nibbling at any house and/or garden plants	No; Yes
Cat nibbling at any house and/or garden plants - which	None; Grass; Other
Any substances added to the water in a vase with cut flowers - what	None; Natural (sugar/salt); Chemicals
Any smokers in household	No; Yes
Carpet cleaned in the last 5-year period	No; Yes
Carpet cleaning - method	None; Home cleaning; Commercial
Carpet cleaning - frequency of cleaning/5 years	0; 1-2/5 years; >2/5 years
Any poisonous substances accidentally eaten by cat	No; Yes
Any human medicines accidentally eaten by cat	No; Yes
Anti-flea collars used regularly	No; Yes
Anti-flea collars - source of purchase	Veterinary Clinic; Shop
Anti-flea collars - frequency of usage/year	0; 1-2/year; >2/year
Anti-flea sprays/spot-on/powders for cats & dogs used regularly	No; Yes
Anti-flea sprays/spot-on/powders for cats & dogs - type of preparations	None; Long acting; Short acting
Anti-flea sprays/spot-on/powders for cats & dogs - frequency of usage/year	0; 1-12/year; >12/year

Table 13d. List of all variables which were found not to be significant when subjected to univariate analyses from cases, random and matched controls cats. All other variables are shown in Tables 9a to 9j and Table 12. First category of all variables was used as a reference category. (GIT = gastrointestinal tract).

Variable	Variable categories
Cat environment - continued	
Anti-flea sprays/spot-on/powders for dogs only used regularly	No; Yes
Anti-flea sprays/spot-on/powders for dogs only - product/brand name	None; Vita Pet spray
Anti-flea sprays/spot-on/powders for dogs only - frequency of usage/year	0; 2/year
Insect growth regulators used regularly	No; Yes
IGR - product/brand name	None; Program; Vet Kem Ovitrol
IGR - frequency of use per year	0; 1-12/year; 24-52/year
Access to any artificial fertilisers stored on property	No; Yes
Owning a hobby/commercial farm and/or livestock	No; Yes
Application of artificial fertilisers	No; Yes
Iodine solutions for farm animals	No; Yes
Access to mineral salt licks/water supplemented with iodine cleaning/disinfecting solutions/ointments/ washes/other medicines with iodine	No; Yes
Cat ever seen licking the salt licks or drinking water with iodine	No; Yes
Ever used any iodine containing agents for treatment of pets including the survey cat	No; Yes
Ever used any iodine containing agents for treatment of pets including the survey cat - product/brand name	None; Iodine tincture
Cat diet	
Person feeding the cat most of the time	Self; Other; Both/everyone
Frequency of feeding each day	1-5/day; Food available 24 hours
Special diet for medical reasons	No; Yes
Brand/name of medical diet	Prescription; Non prescription diet
Length of being on special diet full time (0-36 months)	Up 12 months; > 12 months
Length of being on special diet full time before hyperthyroidism diagnosis (0-27 months)	Up 12 months; > 12 months
Brand of dry food - Whiskas	No; Yes
Brand of dry food - Friskies Go Cat	No; Yes
Brand of dry food - Whiskettes	No; Yes
Brand of dry food - Hill's Prescription Diets	No; Yes
Brand of dry food - Iams Eukanuba	No; Yes
Brand of dry food - Pro Visions Pro Plan	No; Yes
Brand of dry food - KiteKat	No; Yes
Brand of dry food - Purina	No; Yes
Other dry food brands	No; Yes
Flavour of dry food - variety	No; Yes
Flavour of dry food - fish (ocean [tuna, sardines, pilchards, mackerel] & ocean/freshwater [salmon], and seafood [prawn])	No; Yes
Flavour of dry food - turkey/duck	No; Yes
Flavour of dry food - beef/veal	No; Yes

Table 13e. List of all variables which were found not to be significant when subjected to univariate analyses from cases, random and matched controls cats. All other variables are shown in Tables 9a to 9j and Table 12. First category of all variables was used as a reference category. (GIT = gastrointestinal tract).

Variable	Variable categories
Cat diet - continued	
Flavour of dry food - lamb/mutton	No; Yes
Flavour of dry food - game - venison	No; Yes
Flavour of dry food - cheese	No; Yes
Flavour of dry food - vegetables	No; Yes
Flavour of dry food - milk	No; Yes
Other flavours of dry food	No; Yes
Canned cat food	No; Yes
Brand of canned food - Chef	No; Yes
Brand of canned food - Whiskas	No; Yes
Brand of canned food - canned fish for human consumption	No; Yes
Brand of canned food - Friskies Fancy Fiest/Buffet	No; Yes
Brand of canned food - Gourmet	No; Yes
Brand of canned food - Dine	No; Yes
Brand of canned food - Iams	No; Yes
Brand of canned food - Rival	No; Yes
Brand of canned food - Garfield	No; Yes
Brand of canned food - Hill's Prescription Diets	No; Yes
Other brands of canned cat food	No; Yes
Gourmet canned food (Friskies Fancy Fiest/Buffet/Gourmet/Dine/Iams	No; Yes
Flavour of canned food - jelly meat	No; Yes
Flavour of canned food - casserole	No; Yes
Flavour of canned food - chicken	No; Yes
Flavour of canned food - turkey/duck	No; Yes
Flavour of canned food - beef/veal meat	No; Yes
Flavour of canned food - rabbit	No; Yes
Flavour of canned food - egg	No; Yes
Pet roll for cats or for dogs & cats	No; Yes
Daily proportion of pet roll	0; Up ¼; > ¼
Pottle (cat food in plastic tub container)	No; Yes
Daily proportion of pottle	0; Up ¼; > ¼
Dog food	No; Yes
Daily proportion of dog food	0; Up ¼; > ¼
Daily proportion of fresh raw meat, offal, fish	0; Up ¼; > ¼
Type of fresh raw meat - sheep meat (lamb, mutton, sheep skirting, mince, sheep fat trimmings)	No; Yes
Type of fresh raw meat - sheep offal (liver, kidney, heart, lungs)	No; Yes
Type of fresh raw meat - chicken meat	No; Yes
Type of fresh raw meat - chicken bones	No; Yes
Daily proportion of dairy products	0; Up ¼;
Daily proportion of cooked meat - dinner leftovers	0; Up ¼; > ¼
Grease/gravy from pan (dinner leftovers)	No; Yes

Table 13f. List of all variables which were found not to be significant when subjected to univariate analyses from cases, random and matched controls cats. All other variables are shown in Tables 9a to 9j and Table 12. First category of all variables was used as a reference category. (GIT = gastrointestinal tract).

Variable	Variable categories
Cat diet - continued	
Delicatessen products (small goods for human consumption)	No; Yes
French fries (chips), savoury chips (chippies), cereals, sweets (biscuits), sweet pastry, bread (cat "junk" food)	No; Yes
Weekly/monthly proportion of cat "junk" food	0; Once every 2 weeks or less; Daily to once/week
Cat treats	No; Yes
Weekly/monthly proportion of cat treats	0; Once every 2 weeks or less; Daily to once/week
Name/type of cat treats	None; Love hearts; Other
Extra things added to cat food - laxatives	No; Yes
Other source(s) of food - from neighbours	No; Yes
Other source(s) of food - from rubbish bins	No; Yes
Type of prey - rats	No; Yes
Type of prey - rabbits	No; Yes
Type of prey - birds	No; Yes
Type of prey - insects - moths, cicadas, crickets, cockroaches, flies, prying mantis	No; Yes
Type of prey - frogs/lizards	No; Yes
Seen frequency of liquid drinking	Never; Occasionally; Frequently
Tap water - artesian bore (in cat dish)	No; Yes
Tap water - city supply (in cat dish)	No; Yes
Tap - filtered water (in cat dish)	No; Yes
Tap - purified water (in cat dish)	No; Yes
Tap - other water (in cat dish)	No; Yes
Other water sources - sink	No; Yes
Other water sources - shower	No; Yes
Other water sources - toilet	No; Yes
Other water sources - flower vase	No; Yes
Other water sources - flower pot saucers	No; Yes
Other water sources - garden pond	No; Yes
Other water sources - swimming/spa pools	No; Yes
Other water sources - animals troughs	No; Yes
Other sources of water	No; Yes
Type of milk	Full cream; Homogenised; Other (including soy milk and animal milk replacements)
Type of cat food serving dish - plastic	No; Yes
Type of cat food serving dish - enamel	No; Yes
Type of cat food serving dish - aluminum	No; Yes
Type of cat food serving dish - stainless steel	No; Yes
Type of cat food serving dish - crockery	No; Yes
Exposure of food to direct sunlight (>2 h)	No; Yes
Cleaning of cat serving dish	No; Yes
Method/solution used for dish cleaning - brush/scraper	No; Yes

Table 13g. List of all variables which were found not to be significant when subjected to univariate analyses from cases, random and matched controls cats. All other variables are shown in Tables 9a to 9j and Table 12. First category of all variables was used as a reference category. (GIT = gastrointestinal tract).

Variable	Variable categories
Cat diet - continued	
Method/solution used for dish cleaning - dishwasher	No; Yes
Dry food package size (grams)	Up 1000g; 1001-15000g
Dry food storage before opening - where	Not stored; Pantry/cupboard; Laundry/garage/shed/cattery; Bench
Dry food before opening - how long (days)	0-7 days; 8-90 days
Dry food after opening - where	Not stored; Pantry/cupboard/ laundry/garage/shed/cattery/fridge not in the original container/box/ bag; Pantry/cupboard; Laundry/ garage/shed/cattery/fridge; Bench
Dry food storage after opening - how long (days)	1-21 days; 22-120 days
Canned food package size (grams)	85-410g; 411-800g
Canned food storage before opening - where	Not stored; Pantry/cupboard; Laundry/garage/shed/cattery;Bench
Canned food storage before opening - how long (days)	0-7 days; 8-90 days
Canned food storage after opening - where	Not stored;Fridge; Pantry/cupboard
Canned food storage after opening - how long (days)	0-3 days; 4-7 days
Pet roll for dogs and cats or dogs package size (grams)	200-750g; 751-3000g
Pet roll storage before opening - where	Not stored; Pantry/cupboard; Fridge/freezer
Pet roll storage before opening - how long (days)	0-7 days; 8-90 days
Pet roll storage after opening - where	Not stored;Pantry/cupboard; Fridge
Pet roll storage after opening - how long (days)	1-3 days; 4-14 days
Pottle size (grams)	300-500g; 501-1000g
Pottle storage before opening - where	Not stored; Fridge/freezer
Pottle storage before opening - how long (days)	0-3 days; 4-7 days
Pottle storage after opening - where	Not stored; Fridge
Pottle storage after opening - how long (days)	0-3 days; 4-14 days
Raw fresh meat - portion/package size (grams)	20-500g; 501-5000g
Raw fresh meat storage before opening - where	Not stored; Fridge; Freezer
Raw fresh meat storage before opening - how long (days)	0-3 days; 4-180 days
Raw fresh meat storage after opening - where	Not stored; Fridge
Raw fresh meat storage after opening - how long (days)	0-3 days; 4-8 days
Cat treats - size (grams)	100g; 250g
Cat treats storage before opening - where	Not stored; Pantry/cupboard;Bench
Cat treats storage before opening - how long (days)	0-7 days; 8-60 days
Cat treats storage after opening - where	Not stored; Pantry/cupboard;Bench
Cat treats storage after opening - how long (days)	5-14 days; 15-120 days
Comments about feeding - food which cat likes	No; Yes
Comments about feeding - food which cat dislikes	No; Yes
Comments about feeding - food which upsets cat GIT after eating it - loose feaces/vomiting/flatulence	No; Yes
Comments on cat eating habits	No; Yes

Appendix 6

New Zealand cat study - hyperthyroidism - cat owner questionnaire

Veterinary practice's address:

C (clinical case)
MC (match control)
RC (random control)

For office use only

NEW ZEALAND CAT STUDY - HYPERTHYROIDISM

For CONTROL CAT (non hyperthyroid) history for the last 3years

For HYPERTHYROID CAT history for the 3-year period preceding the diagnosis of hyperthyroidism

OWNER'S NAME: .....

ADDRESS (include residential address also): .....

.....

TELEPHONE NO: .....

CAT'S NAME: .....

CURRENT DATE: .....

Please answer each question by putting a tick in the appropriate box(es), or by writing in your answer in the space provided. When you are asked to write a number, please write the number, even if it is "0". You are welcome to contact your cat's veterinary clinic to ask some details.

---

1. Your residence type:

☐ city ☐ town ☐ country non-farm

☐ commercial farm ☐ hobby farm (lifestyle block)

→ *state* type (e.g.: dairy, sheep and beef, crops): .....

---

2. How many times have you moved house, together with your cat, within the last 5-year period: [.....].

How long have you lived at the present address: [.....] years [.....] months

---

3. In your household, how many people, including yourself, are:

☐ adult females ☐ adult males ☐ children under 18

---

4. Are there any human members of the household who suffer from hyperthyroidism:

☐ yes ☐ no ☐ don't know

→ *specify* how many are:

☐ adult females ☐ adult males ☐ children females under 18

☐ children males under 18

YOUR CAT

5. How old is your cat: [.....] years [.....] months [.....] don't know
Write the approximate birth date of your cat, if known: .....

---

6. How long have you owned your cat: [.....] years [.....] months
What was the source of your cat (e.g.: from breeder, RSPCA, pet shop, etc.): .....
.....

---

7. What breed is your cat: pure breed [ ] mixed breed [ ]
If Pure-bred, state breed and short breeding history:.....
.....

---

8. Does your cat have long (e.g.: Persian) hair: yes [ ] no [ ]

---

9. What sex is your cat:

☐ castrated male ☐ spayed female
☐ entire male ☐ entire female ☐ don't know

go to question 11

---

10. How old was your cat when it was desexed :
[.....] years [.....] months [.....] don't know

YOUR CAT'S MEDICAL HISTORY

11. How many times has the cat been to the vet over the last 3 years: .....
On how many occasions was this for vaccination: .....

---

12. Has your cat been vaccinated against the following viral diseases:

	Yes	No	Don't know
Snuffles/feline enteritis (routine)	[ ]	[ ]	[ ]
Chlamydia (additional)	[ ]	[ ]	[ ]
Leukaemia (additional)	[ ]	[ ]	[ ]

How often do you vaccinate your cat (write the vaccine name - symbol - if you know): .....

---

13. How often do you worm your cat: .....
What worming preparations do you use for your cat: .....
.....
.....

14. During the last 3 years, has your cat suffered from any of the following medical conditions:

	Yes	No	Don't know	
Cat fight wound	[ ]	[ ]	[ ]	☐
Dental/gum disease	[ ]	[ ]	[ ]	☐
Respiratory tract disease: snuffles (discharge from nose and/or runny eyes)	[ ]	[ ]	[ ]	☐
Urinary tract problems("bladder" problems)	[ ]	[ ]	[ ]	☐
Thyroid gland problems	[ ]	[ ]	[ ]	☐
Allergy: skin	[ ]	[ ]	[ ]	☐
food	[ ]	[ ]	[ ]	☐
Bowel (gut) problems	[ ]	[ ]	[ ]	☐
Diarrhoea	[ ]	[ ]	[ ]	☐
Periods of self-starvation (not eating at all)	[ ]	[ ]	[ ]	☐
Poisoning (toxicity)	[ ]	[ ]	[ ]	☐
Cancer (tumour)	[ ]	[ ]	[ ]	☐
Other illnesses now and in the past - specify:	[ ]	[ ]	[ ]	☐

.....

.....

Any special comments on your cat's health or behaviour now and in the past: ☐☐☐☐

.....

.....

.....

---

15. Has your cat been tested for:

	Yes	No	Don't know	Recent test result positive	Recent test result negative	
Feline leukaemia virus	[ ]	[ ]	[ ]	[ ]	[ ]	☐☐
Feline immunodeficiency virus (cat "AIDS")	[ ]	[ ]	[ ]	[ ]	[ ]	☐☐

The questions 16 - 18 should be answered by owners of hyperthyroid cat only

16. When was the diagnosis of hyperthyroidism made (write the date or cat's age at diagnosis): ..... ☐☐☐

17. Which of the following clinical signs did you notice your cat exhibiting before and/or at the time of hyperthyroidism diagnosis:

	YES	NO	DON'T KNOW
Weight loss	[ ]	[ ]	[ ]
Increased appetite	[ ]	[ ]	[ ]
Increased activity	[ ]	[ ]	[ ]
Increased restlessness	[ ]	[ ]	[ ]
Increased irritability	[ ]	[ ]	[ ]
Resenting handling	[ ]	[ ]	[ ]
Increased thirst	[ ]	[ ]	[ ]
Increased urination	[ ]	[ ]	[ ]
Diarrhoea	[ ]	[ ]	[ ]
Greasy, pale stools	[ ]	[ ]	[ ]
Increased stools	[ ]	[ ]	[ ]
Vomiting	[ ]	[ ]	[ ]
Avoid warm places	[ ]	[ ]	[ ]
Panting	[ ]	[ ]	[ ]
Muscle weakness	[ ]	[ ]	[ ]
Muscle shaking	[ ]	[ ]	[ ]
Rapid growth of nails	[ ]	[ ]	[ ]
Poor/unkempt hair coat	[ ]	[ ]	[ ]
Excessive hair shedding	[ ]	[ ]	[ ]
Excessive grooming	[ ]	[ ]	[ ]
Localised loss of hair	[ ]	[ ]	[ ]
Reduced activity	[ ]	[ ]	[ ]
Reduced appetite	[ ]	[ ]	[ ]
Very lazy and sleepy	[ ]	[ ]	[ ]
Increased meowing	[ ]	[ ]	[ ]
Other signs - specify	[ ]	[ ]	[ ]

.....

At the time of diagnosis did you notice anything else unusual in your cat's health, behaviour, appearance: .....

.....

---

18. What kind of treatment has your cat received for hyperthyroidism:

	Yes	No	Don't know
Drug (pill)	[ ]	[ ]	[ ]
Surgical (operation)	[ ]	[ ]	[ ]
Radioactive Iodine	[ ]	[ ]	[ ]
None	[ ]	[ ]	[ ]

What was the final outcome of the treatment:

Alive and well	[ ]	Alive and unwell	[ ]
Euthanasia	[ ]	Died	[ ]

Why did you choose that particular treatment option: .....

.....

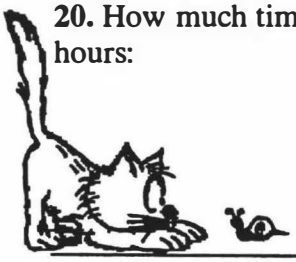
YOUR CAT'S ENVIRONMENT

While answering the following questions (**19 - 48**), please think about your cat's territory (the areas it normally uses) inside your home and outside. How big is the territory (including your property and the neighbourhood)? .....

19. What is in your cat's territory (you can *tick* more than 1 box):

- ☐ lawn
- ☐ garden
- ☐ orchard
- ☐ pasture
- ☐ crop field
- ☐ park
- ☐ playground
- ☐ industrial area
- ☐ other: .....

20. How much time does your cat usually spend outside (in the open air) every 24 hours:



- None [ ]
- Less than 1 hour a day [ ]
- 1-3 hours in a day [ ]
- 3-12 hours in a day [ ]
- 12-24 hours in a day [ ]

21. Do you let your cat out whenever it wants to go: Yes [ ] No [ ]

22. Where does your cat sleep at night:
- Inside your house [ ]
- Outside your house [ ]
- Other - specify: .....

23. Please describe the type of bedding on which your cat spends most of its time; if it is commercial bedding write brand and manufacturer of it: .....
.....
.....

24. Do you clean your cat's bedding:

☐ yes ☐ no ☐ not applicable

↘ how often and what do you use for cleaning/washing: .....
.....

25. Do you provide indoor (inside your house, garage) toilet facilities, e.g. litter tray, for your cat:

❒ yes ❒ no

↘ *specify* the type and if it is commercial litter write brand and manufacturer: .....

---

26. Do you use sawdust and/or wood shavings for bedding and/or for litter trays:

❒ yes ❒ no ❒ not applicable

↘ *go to question 27* ↘ *go to question 27*

↘ *give* source of shavings/sawdust .....

.....

↘ is the wood treated with preservatives:

❒ yes ❒ no ❒ don't know

---

27. How many cats are/were in your household for the last **5**-year period:

❒ 1 ❒ more than 1

↘ *go to question 29* ↘ *write* the number and *give* details whether related or not: .....

.....

---

28. Are you aware of any of these other cats in your household suffering from hyperthyroidism **now** or in the **past**:

❒ yes ❒ no ❒ don't know

↘ *go to question 29* ↘ *go to question 29*

↘ the number of the hyperthyroid cat(s) not including the survey cat:[.....]

↘ how old were these other cats at the time of their hyperthyroidism diagnosis:.....

.....

↘ are these other hyperthyroid cat(s) related to the cat under survey:

❒ yes ❒ no ❒ don't know

↘ *specify* the relationship, if it is possible: .....

.....

29. Do you own any other pets:

❒ yes ❒ no

↘ *specify*: .....

.....

30. Have you <u>ever</u> left your cat in a boarding cattery:

❒ yes ❒ no ❒ don't know

↘ how many times a year: .....

for how long: .....days. .....weeks .....months

31. Do you take your cat to cat shows:

❒ yes ❒ no

↘ how many times a year do you show your cat: .....

32. How would you describe your cat's weight at present:

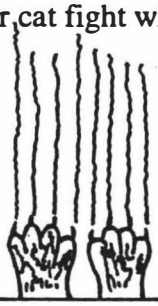
Normal weight for age [ ]
Heavier than normal [ ]
Lighter than normal [ ]

Current weight: .....(kg)

33. How would you describe your cat's <u>every day</u> behaviour (currently) remembering that certain breeds show different patterns of behaviour:

Normal [ ]
Active/energetic [ ]
Hyperactive [ ]
Lazy [ ]
Very lazy [ ]
Don't know [ ]

34. Does your cat fight with other cats (as far as you know):



Constantly [ ]
Frequently [ ]
Occasionally [ ]
Seldom [ ]
Never [ ]
Don't know [ ]

35. Do you use and/or are you aware of any chemical substances used within your cat's territory, on lawn, garden, orchard, paddock, crop field, park, street, etc.:

	Yes	No	Don't know
Manure	[ ]	[ ]	[ ]
Artificial fertilisers (used to make plants grow)	[ ]	[ ]	[ ]
Herbicides (used to kill weeds)	[ ]	[ ]	[ ]
Pesticides (used for killing pests, especially insects)	[ ]	[ ]	[ ]
Fungicides (used to kill moulds on trees and plants)	[ ]	[ ]	[ ]
Snail and slug baits	[ ]	[ ]	[ ]
Pest control poisons (for rats, mice, possums, rabbits)	[ ]	[ ]	[ ]

Other - specify: .....

If you answer **Yes** to any substances listed above, please provide (if possible) the following details:

Name of product(s): .....

Active ingredient(s): .....

Manufacture of the product(s): .....

How many times per year the product(s) are used: .....

.....

---

36. Do you use, the following chemicals, indoors:

	Yes	No	How many times per year
Fly spray	[ ]	[ ]	.....
Ant killers	[ ]	[ ]	.....
Flea control in house	[ ]	[ ]	.....
Indoor plants fertilisers	[ ]	[ ]	.....
Chemical sprays on your indoor plants	[ ]	[ ]	.....
Baits for pests	[ ]	[ ]	.....

Other - specify: .....

If you answer **Yes** to any substances listed above, please provide (if possible) the following details:

Name of product(s): .....

Active ingredient(s): .....

Manufacture of the product(s): .....

---

37. Does your cat nibble at any house and/or garden plants:

❒ yes ❒ no ❒ don't know

↘ *specify* .....

---

38. Do you add anything to the water in a vase with cut flowers:

❒ yes ❒ no ❒ don't know

↘ *specify* .....

39. In your home, how many people, including yourself, are smokers: [.....] ☐☐

---

40. Have you had your carpet cleaned in the last **5**-year period: ☐

❒ yes ❒ no ❒ don't know ☐

↘ how many times, what chemical preparations were used, what method, ☐

e.g.: wet, dry, steam extraction, commercial, home cleaning: .....

.....

.....

---

41. Are you aware of accidental eating by your cat (direct and indirect - by grooming, e.g.: licking paint, tar off its coat) any possibly poisonous substances: ☐

❒ yes ❒ no ❒ don't know ☐

↘ *specify* ..... ☐

.....

---

42. Are human medicines, such as pain killers, disinfectants (e.g.: aspirin, paracetamol, iodine solutions) or other used to treat your cat, if it is sick: ☐

❒ yes ❒ no ❒ don't know ☐

↘ *specify* ..... ☐

.....

---

43. Do you regularly use, either:

❒ on your cat ❒ on cat's bedding ❒ on both ☐

the following anti-flea products:	Yes	No	Don't know	
Collars:	[ ]	[ ]	[ ]	☐☐☐
Brand(s):				
How many times per year:				
Sprays, spot-on or **powders**, which are designed for cats or cats and dogs:	[ ]	[ ]	[ ]	☐☐☐
Brand(s):				
How many times per year:				
Sprays, spot-on or **powders**, which are designed for dogs only:	[ ]	[ ]	[ ]	☐☐☐
Brand(s):				
How many times per year:				
IGR (insect growth regulators) e.g.: Program	[ ]	[ ]	[ ]	☐☐☐

---

44. Has your cat got access to any artificial fertilisers stored on your property:

❒ yes ❒ no ❒ don't know

↘ *write* the names of the fertilisers stored on your property: .....

.....

If you do not have a farm or any livestock, go to question 49

45. What method of artificial fertiliser application do you use:

❒ tractor/spreader ❒ truck ❒ aerial

---

46. Do you provide mineral salt licks to your farm animals:

❒ yes ❒ no ❒ don't know

↘ *specify* ingredients/brand(s): .....

.....

---

47. Do you use any iodine solutions to supplement iodine levels in your farm animals:

❒ yes ❒ no ❒ don't know

↘ *specify* .....

---

48. Has your cat got access to mineral salt licks and/or to water supplemented with iodine and/or to cleaning/disinfecting solutions/ointments/washes or other medicines containing iodine:

❒ yes ❒ no ❒ not applicable

↘ do you ever see your cat licking the salt lick or drinking that water:

❒ yes ❒ no

Have you ever used any iodine containing agents for treatment of your pet(s), including the survey cat:

❒ yes ❒ no

↘ *specify*.....

.....

YOUR CAT'S DIET

49. Who feeds your cat most of the time: Self [ ]
Other person [ ]

50. How often is your cat fed each day:

Once [ ]
Twice [ ]
Three or more [ ]
Food available all the time [ ]

51. Is your cat on a special diet for medical reasons:

❒ yes ❒ no ❒ don't know

↘ *specify* .....

.....

52. Estimate the daily proportion (*circle* or *tick* anywhere on the line) of the following food in your cat's diet. Write the brand in the left column (e.g.: Chef, KiteKat, Iams-Eukanuba, etc.). Write the flavour in the right column (e.g.: fish, chicken, liver, etc.).

Example: You give your cat 1/3 of <u>its daily food intake</u> as dry cat food, 1/3 canned cat food and 1/3 fresh raw meat (gravy beef). You circle or tick on the scale '1/3' for each type of food.

Brand		**Flavour**
	Dry cat food (biscuits)	
.....		.....
.....	none ¼ ½ ¾ all	.....
.....		.....
.....		.....
	Canned cat food	
.....		.....
.....	none ¼ ½ ¾ all	.....
.....		.....
.....		.....
	Pet roll for <u>cats</u> or for <u>dogs and cats</u>	
.....		.....
.....	none ¼ ½ ¾ all	.....
.....		.....
.....		.....
	Cat food in plastic tub (pottle) container	
.....		.....
.....	none ¼ ½ ¾ all	.....
.....		.....
.....		.....

Dog food

Specify type/brand | **Specify type/brand**

..... | .....

..... none ¼ ½ ¾ all .....

..... | .....

..... | .....

Fresh raw meat (e.g.: gravy beef, pure beef), offal (kidney, liver, heart, lung)

Specify type | **Specify type**

..... | .....

..... none ¼ ½ ¾ all .....

..... | .....

..... | .....

How often and how much do you give to your cat other food types (e.g.: dairy products (cheese, butter, ice cream), eggs, cooked meat, fat trimmings, raw/cooked fish, grease/gravy from pan, delicatessen products (small goods), vegetables, fruits, cereal, sweets, nuts, chips, savoury chippies, cat treats (fishys, love hearts, milk drops, kluckers), other)

Specify type **Times fed per week** **How much** (cupfuls)

.....

.....

.....

.....

.....

.....

---

53. Do you add anything to your cat's food, e.g.: vitamins, minerals, salt, kelp:

❒ yes ❒ no

↘ *specify* including name, brand, how often; if it is salt, indicate if it is iodised: .....

.....

---

54. Does your cat obtain food other than what you feed it, e.g.: prey (mice, birds, moths, cicadas other small animals), material from rubbish bins:

❒ yes ❒ no ❒ don't know

↘ *specify* .....

---

55. Do you see your cat drinking any liquids:

Frequently [ ]
Occasionally [ ]
Never [ ]

---

56. How would you describe the quantities of liquid (milk and/or water) your cat drinks each day:

Excessive [ ]
Normal [ ]
Minimal [ ]
Don't know [ ]

---

57. What type of water does your cat drink (you can *tick* more than 1 box):

Tap water (in cat's dish): [ ]

- ☐ rain water
- ☐ artesian bore
- ☐ city supply
- ☐ filtered
- ☐ purified
- ☐ other: .....

Other: [ ]

- ☐ sink
- ☐ bath
- ☐ shower
- ☐ toilet
- ☐ flower vases
- ☐ flower pot saucers
- ☐ puddles
- ☐ garden pond
- ☐ swimming/spa pools
- ☐ animals' troughs
- ☐ other: .....

---

58. If your cat drinks milk, specify type: .....

---

59. What type of dish, if any, do you use to serve your cat's food:

- ☐ plastic
- ☐ enamel
- ☐ aluminium
- ☐ stainless steel
- ☐ crockery
- ☐ other: .....

---

60. How long, on average, does the food stay in the dish :

Dry food (biscuits): .....min. .....hours .....days
Other than dry: .....min. .....hours .....days .

---

61. If the food stays in the dish more than 2 hours, is the dish with food left in direct sunlight:

Yes [ ]
No [ ]
Not applicable [ ]

---

62. Do you clean your cat's serving dish:

☐ yes ☐ no ☐ don't know

↘ how often and what do you use for cleaning: .....

.....

63. Do you use the microwave to warm your cat's meals:

❒ yes ❒ no ❒ don't know

↘ how often do you do that: .....

64. If you feed commercial pet food describe briefly, where and how long do you store it before and after opening:

	Pack -age size	Before opening		After opening	
		Where	How long (days)	Where	How long (days)
Dry					
Canned					
Pet roll					
Pottle					
Fresh					
Cat treats					

Appendix 7

New Zealand cat study - hyperthyroidism - cat veterinarian questionnaire

Veterinary practice's address:

C (clinical case)

For office use only

NEW ZEALAND CAT STUDY - HYPERTHYROIDISM

MEDICAL HISTORY FOR THE 3-YEAR PERIOD PRECEDING THE DIAGNOSIS OF HYPERTHYROIDISM

OWNER'S NAME: .....

ADDRESS (include residential address also): .....

.....

PHONE NO: .....

CURRENT DATE: .....

CAT'S: NAME **Current AGE or DOB** **BREED** **COLOUR** **SEX**

.....

DATE OF DIAGNOSIS (cat's age at diagnosis): .....

1. How many times has the cat been to the vet over the last 3 years : .....
On how many occasions was this for vaccination: .....

2. Vaccination:

	Yes	No	Don't know
Panleukopenia	[ ]	[ ]	[ ]
Herpesvirus	[ ]	[ ]	[ ]
Calicivirus	[ ]	[ ]	[ ]
Chlamydia	[ ]	[ ]	[ ]
Leukaemia	[ ]	[ ]	[ ]

3. Anti-worm treatment:

Preparation	Dose	Date
.....		
.....		
.....		
.....		
.....		
.....		

4. Tests and results:	Yes	No	Result (+ve or –ve)	
FeLV	[ ]	[ ]	.....	☐☐
FIV	[ ]	[ ]	.....	☐☐
Other - specify	[ ]	[ ]	.....	☐☐

.....

.....

.....

5. Clinical signs at the time of presentation:

	Yes	No	
Weight loss	[ ]	[ ]	☐
Polyphagia	[ ]	[ ]	☐
Hyperactivity	[ ]	[ ]	☐
Restlessness	[ ]	[ ]	☐
Irritability	[ ]	[ ]	☐
Resenting clinical examination	[ ]	[ ]	☐
Polydipsia	[ ]	[ ]	☐
Polyuria	[ ]	[ ]	☐
Diarrhoea	[ ]	[ ]	☐
Steatorrhoea	[ ]	[ ]	☐
Increased faecal volume	[ ]	[ ]	☐
Increased incidence of vomiting	[ ]	[ ]	☐
Heat intolerance	[ ]	[ ]	☐
Polypnoea (panting)	[ ]	[ ]	☐
Muscle weakness	[ ]	[ ]	☐
Muscle tremor	[ ]	[ ]	☐
Rapid growth of nails	[ ]	[ ]	☐
Poor/unkempt hair coat	[ ]	[ ]	☐
Excessive hair shedding	[ ]	[ ]	☐
Excessive grooming	[ ]	[ ]	☐
Alopecia	[ ]	[ ]	☐
Reduced activity	[ ]	[ ]	☐
Reduced appetite	[ ]	[ ]	☐
Depression	[ ]	[ ]	☐
Tachycardia	[ ]	[ ]	☐
Heart murmur	[ ]	[ ]	☐
Arrhythmias	[ ]	[ ]	☐
Abnormally strong femoral pulse	[ ]	[ ]	☐
Mild pyrexia/intermittent fever	[ ]	[ ]	☐
Hyperaemia of MM & skin	[ ]	[ ]	☐
ECG changes	[ ]	[ ]	☐
Congestive heart failure (CHF)	[ ]	[ ]	☐
Palpable thyroid gland	[ ]	[ ]	☐
Other - specify	[ ]	[ ]	☐

.....
.....
Comments: .....
.....
.....
.....
.....

	Result	Date
Thyroid function tests:		
Total T_4	.....	
Total T_3	.....	

Other - specify: .....
.....
.....

---

6. Treatment:

	Yes	No
Medical	[ ]	[ ]
Surgical	[ ]	[ ]
Radioactive Iodine I^{131}	[ ]	[ ]
None	[ ]	[ ]

---

7. Outcome:

Alive and well	[ ]	Alive and unwell	[ ]
Euthanasia	[ ]	Died	[ ]

---

8. Histopathology of thyroid gland:

Yes	No	Result
[ ]	[ ]	.....

---

9. Have you <u>ever</u> used or prescribed any of the following <u>iodine</u> containing agents for treatment of this cat:

	Yes	No
Surgical scrub	[ ]	[ ]
Antiseptic solution (before and after surgery)	[ ]	[ ]
Disinfectant	[ ]	[ ]
Tincture spray	[ ]	[ ]
Cream/ointment	[ ]	[ ]
Wash/shampoo	[ ]	[ ]
Radiocontrast media	[ ]	[ ]
Other - specify	[ ]	[ ]

.....
.....
.....
.....

10. Have you <u>ever</u> used any of the following drugs for treatment of this cat?

	Yes	No
Corticosteroids: short acting	[ ]	[ ]
long acting	[ ]	[ ]
Antibiotics including sulfonamides - specify	[ ]	[ ]
Anaesthetic agents - specify	[ ]	[ ]
Anticonvulsants - specify	[ ]	[ ]
Diuretics - specify	[ ]	[ ]
Hormones - specify	[ ]	[ ]
Aspirin	[ ]	[ ]
Other - specify	[ ]	[ ]

.....

.....

.....

.....

If you answer **Yes** to any remedies listed above, please provide the relevant details in the appendix.

APPENDIX

10. Cat's visits at the veterinary clinic:

Reason for visit: ....
Date of visit: ....
Diagnosis (if made): ....
Drug(s) administered: ....
....
Surgery performed: ....
Preparation of the surgery field: ....
....
Anaesthetics used: ....
....

Reason for visit: ....
Date of visit: ....
Diagnosis (if made): ....
Drug(s) administered: ....
....
Surgery performed: ....
Preparation of the surgery field: ....
....
Anaesthetics used: ....
....

Reason for visit: ....
Date of visit: ....
Diagnosis (if made): ....
Drug(s) administered: ....
....
Surgery performed: ....
Preparation of the surgery field: ....
....
Anaesthetics used: ....
....

Reason for visit: ....
Date of visit: ....
Diagnosis (if made): ....
Drug(s) administered: ....
....
Surgery performed: ....
Preparation of the surgery field: ....
....
Anaesthetics used: ....
....

Reason for visit: ....
Date of visit: ....
Diagnosis (if made): ....
Drug(s) administered: ....
....
Surgery performed: ....
Preparation of the surgery field: ....
....
Anaesthetics used: ....
....

Reason for visit: ....
Date of visit: ....
Diagnosis (if made): ....
Drug(s) administered: ....
....
Surgery performed: ....
Preparation of the surgery field: ....
....
Anaesthetics used: ....
....

Reason for visit: ....
Date of visit: ....
Diagnosis (if made): ....
Drug(s) administered: ....
....
Surgery performed: ....
Preparation of the surgery field: ....
....
Anaesthetics used: ....
....

Reason for visit: ....
Date of visit: ....
Diagnosis (if made): ....
Drug(s) administered: ....
....
Surgery performed: ....
Preparation of the surgery field: ....
....
Anaesthetics used: ....
....

Appendix 8

SPSS codes (two examples only) for descriptive methods (crosstabulation and frequencies)

Cat and owner factors

```
CROSSTABS
 /TABLES=rsdctrc move012c nowcatc f3 m3c ch3c thh4c thf4 thm4
ownl12 ownl6 owns brdslsio hair8 dsexya BY srvtype
 /FORMAT= AVALUE TABLES
 /CELLS= COUNT COLUMN .
```

Cat age distribution at the time of hyperthyroidism diagnosis

```
FREQUENCIES
 VARIABLES=dgage16
 /STATISTICS=STDDEV RANGE MINIMUM MAXIMUM SEMEAN
MEAN MEDIAN
 /HISTOGRAM NORMAL
 /ORDER ANALYSIS .
```

SPSS codes (one example only) for univariate analysis, used for screening case and random controls data sets for potential risk factors

```
LOGISTIC REGRESSION VAR=srvtyper
 /SELECT srvtypec NE 2
 /METHOD=ENTER thc4
 /CONTRAST (thc4)=Indicator(1)
 /PRINT=CI(90)
 /CRITERIA PIN(.05) POUT(.10) ITERATE(20) CUT(.5) .
```

SPSS code for forward stepwise multiple unconditional logistic regression analysis for all significant variables "*" (from univariate analysis, see Tables 9a to 9j) for case (1) - random control (0) status

Cat and owner factors

```
LOGISTIC REGRESSION VAR=srvtyper
 /SELECT srvtypec NE 2
 /METHOD=FSTEP(LR)  move012c nowcatc ch3c thf4 age3y3c2 ownl12
ownoth6 owns brdslsio sex dsexya
 /CONTRAST (move012c)=Indicator(1) /CONTRAST
(nowcatc)=Indicator(1) /CONTRAST (ch3c)=Indicator(1) /CONTRAST
 (thf4)=Indicator(1) /CONTRAST (age3y3c2)=Indicator(1) /CONTRAST
(ownl12)=Indicator(1) /CONTRAST (ownoth6)=Indicator(1)
/CONTRAST (owns)=Indicator(1) /CONTRAST (brdslsio)=Indicator (1)
/CONTRAST (sex)=Indicator(1) /CONTRAST (dsexya)=Indicator(1)
 /PRINT=CI(95)
 /CRITERIA PIN(.05) POUT(.10) ITERATE(20) CUT(.5) .
```

Cat medical history

```
LOGISTIC REGRESSION VAR=srvtyper
 /SELECT srvtypec NE 2
 /METHOD=FSTEP(LR)  fcvrpc12 felvc12 denc14 rtc14 utbc14 gitc14
drhc14 starvc14
 /CONTRAST (fcvrpc12)=Indicator(1) /CONTRAST
(felvc12)=Indicator(1) /CONTRAST (denc14)=Indicator(1) /CONTRAST
(rtc14)=Indicator(1) /CONTRAST (utbc14 )=Indicator(1) /CONTRAST
(gitc14)=Indicator(1) /CONTRAST (drhc14)=Indicator (1) /CONTRAST
(starvc14)=Indicator(1)
 /PRINT=CI(95)
```

/CRITERIA PIN(.05) POUT(.10) ITERATE(20) CUT(.5) .

Cat indoor and outdoor environment

LOGISTIC REGRESSION VAR=srvtyper

/SELECT srvtypec NE 2

/METHOD=FSTEP(LR) past19 indar19 toutc12 bedfc23 bedovc23 bedwlc23 bclm24w sdutc26 htctc28 wgtc32 bhv33c fgtc34c flec36 pchpc36 flebed flescpc mrlkc46

/CONTRAST (past19)=Indicator(1) /CONTRAST (indar19)=Indicator(1) /CONTRAST (toutc12)=Indicator(1) /CONTRAST (bedfc23)=Indicator(1) /CONTRAST (bedovc23)=Indicator(1) /CONTRAST (bedwlc23)=Indicator(1) /CONTRAST (bclm24w)=Indicator(1) /CONTRAST (sdutc26)=Indicator(1) /CONTRAST (htctc28)=Indicator(1) /CONTRAST (wgtc32)=Indicator(1) /CONTRAST (bhv33c)=Indicator (1) /CONTRAST (fgtc34c)=Indicator(1) /CONTRAST (flec36)=Indicator(1) /CONTRAST (pchpc36)=Indicator(1) /CONTRAST (flebed)=Indicator(1) /CONTRAST (flescpc)=Indicator(1) /CONTRAST (mrlkc46)=Indicator(1)

/PRINT=CI(95)

/CRITERIA PIN(.05) POUT(.10) ITERATE(20) CUT(.5) .

Cat diet and feeding practices

LOGISTIC REGRESSION VAR=srvtyper

/SELECT srvtypec NE 2

/METHOD=FSTEP(LR) alt52 drp52c2 cnp52c2 rm52 dp52 cm52 ad53 fot54c liqq56c trw57 bh57 dclf62r mic63

/CONTRAST (alt52)=Indicator(1) /CONTRAST (drp52c2)=Indicator(1) /CONTRAST (cnp52c2)=Indicator(1) /CONTRAST (rm52)=Indicator(1)

/CONTRAST (dp52)=Indicator(1) /CONTRAST (cm52)=Indicator(1)
/CONTRAST (ad53)=Indicator (1) /CONTRAST (fot54c)=Indicator(1)
/CONTRAST (liqq56c)=Indicator(1) /CONTRAST (trw57)=Indicator(1)
/CONTRAST (bh57)=Indicator(1) /CONTRAST (dclf62r)=Indicator(1)
/CONTRAST (mic63)=Indicator(1)
/PRINT=CI(95)
/CRITERIA PIN(.05) POUT(.10) ITERATE(20) CUT(.5) .

Second part of this analysis - pre-final model for the four risk factor groupings (mentioned above)

LOGISTIC REGRESSION VAR=srvtyper
/SELECT srvtypec NE 2
/METHOD=FSTEP(LR) age3y3c2 brdslsio sex dsexya fcvrpc12 felvc12 denc14
rtc14 starvc14 utbc14 past19 bedfc23 bhv33c fgtc34c flebed cnp52c2
fot54c liqq56c rm52 trw57
/CONTRAST (age3y3c2)=Indicator(1) /CONTRAST (brdslsio)=Indicator(1)
/CONTRAST (sex)=Indicator(1) /CONTRAST (dsexya)=Indicator(1) /CONTRAST
(fcvrpc12)=Indicator(1) /CONTRAST (felvc12)=Indicator(1)
/CONTRAST (denc14)=Indicator(1) /CONTRAST (rtc14)=Indicator(1)
/CONTRAST (starvc14)=Indicator(1) /CONTRAST (utbc14)=Indicator(1)
/CONTRAST (past19)=Indicator(1) /CONTRAST (bedfc23)=Indicator(1)
/CONTRAST (bhv33c)=Indicator(1) /CONTRAST (fgtc34c)=Indicator(1)
/CONTRAST (flebed)=Indicator(1) /CONTRAST (cnp52c2)=Indicator(1)
/CONTRAST (fot54c)=Indicator(1) /CONTRAST (liqq56c)=Indicator(1)
/CONTRAST (rm52)=Indicator(1) /CONTRAST (trw57)=Indicator(1)

```
 /PRINT=CI(95)
 /CRITERIA PIN(.05) POUT(.10) ITERATE(20) CUT(.5) .
```

Third part of this analysis - final model for the four risk factor groupings with interactions

```
LOGISTIC REGRESSION VAR=srvtyper
 /SELECT srvtypec NE 2
 /METHOD=ENTER age3y3c2 brdslsio sex dsexya denc14 bedfc23 flebed
cnp52c2
 /METHOD=FSTEP(LR) age3y3c2*denc14 age3y3c2*cnp52c2
cnp52c2*denc14
 /CONTRAST (age3y3c2)=Indicator(1) /CONTRAST
(brdslsio)=Indicator(1) /CONTRAST (sex)=Indicator(1) /CONTRAST
(dsexya)=Indicator(1) /CONTRAST (denc14)=Indicator(1) /CONTRAST
(bedfc23)=Indicator(1) /CONTRAST (flebed)=Indicator(1) /CONTRAST
(cnp52c2)=Indicator(1)
 /PRINT=CI(95)
 /CRITERIA PIN(.05) POUT(.10) ITERATE(20) CUT(.5) .
```

SPSS code for forward stepwise multiple conditional logistic regression analysis for case (1) - matched control (2) status

Cat and owner factors

```
COXREG
 srvtypec /STATUS=srvtyper(1) /STRATA=triadid
 /CONTRAST (rsdctrc)=Indicator(1) /CONTRAST
(move012c)=Indicator(1) /CONTRAST (nowcatc)=Indicator(1)
/CONTRAST (f3)=Indicator(1) /CONTRAST (m3c)=Indicator(1)
```

/CONTRAST (ch3c)=Indicator(1) /CONTRAST (thh4c)=Indicator(1) /CONTRAST (thf4)=Indicator(1) /CONTRAST (thm4)=Indicator(1) /CONTRAST (ownl12)=Indicator(1) /CONTRAST (ownoth6)=Indicator(1) /CONTRAST (owns)=Indicator(1) /CONTRAST (brdslsio)=Indicator(1) /CONTRAST (hair8)=Indicator(1) /CONTRAST (dsexya)=Indicator(1)

/METHOD=FSTEP(COND) rsdctrc move012c nowcatc f3 m3c ch3c thh4c thf4 thm4 ownl12 ownoth6 owns brdslsio hair8 dsexya

/PRINT=CI(95)

/CRITERIA=PIN(.05) POUT(.10) ITERATE(20) .

Cat medical history

COXREG

srvtypec /STATUS=srvtyper(1) /STRATA=triadid

/CONTRAST (fcvrpc12)=Indicator(1) /CONTRAST (chlamc12)=Indicator(1) /CONTRAST (felvc12)=Indicator(1) /CONTRAST (worf)=Indicator(1) /CONTRAST (cfbac14)=Indicator(1) /CONTRAST (denc14)=Indicator(1) /CONTRAST (rtc14)=Indicator(1) /CONTRAST (utbc14)=Indicator(1) /CONTRAST (algsc14)=Indicator(1) /CONTRAST (algfc14)=Indicator(1) /CONTRAST (gitc14)=Indicator(1) /CONTRAST (drhc14)=Indicator(1) /CONTRAST (starvc14)=Indicator(1) /CONTRAST (toxc)=Indicator(1) /CONTRAST (cac14c)=Indicator(1) /CONTRAST (othc14c)=Indicator(1) /CONTRAST (coml4c)=Indicator(1) /CONTRAST (felvyno)=Indicator(1) /CONTRAST (fivyno)=Indicator(1)

/METHOD=FSTEP(COND) fcvrpc12 chlamc12 felvc12 worf cfbac14 denc14 rtc14 utbc14 algsc14 algfc14 gitc14 drhc14 starvc14 toxc cac14c othc14c com14c felvyno fivyno

/PRINT=CI(95)

/CRITERIA=PIN(.05) POUT(.10) ITERATE(20) .

Cat indoor and outdoor environment

COXREG

srvtypec /STATUS=srvtyper(1) /STRATA=triadid

/CONTRAST (lawn19)=Indicator(1) /CONTRAST (gard19)=Indicator(1) /CONTRAST (orch19)=Indicator(1) /CONTRAST (past19)=Indicator(1) /CONTRAST (crfld19)=Indicator(1) /CONTRAST (park19)=Indicator(1) /CONTRAST (plygr19)=Indicator(1) /CONTRAST (othoi)=Indicator(1) /CONTRAST (toutc12)=Indicator(1) /CONTRAST (loutc21)=Indicator(1) /CONTRAST (slep)=Indicator(1) /CONTRAST (bedbc23)=Indicator(1) /CONTRAST (bedsc23)=Indicator(1) /CONTRAST (bedcc23)=Indicator(1) /CONTRAST (bedbbc23)=Indicator(1) /CONTRAST (bedfc23)=Indicator(1) /CONTRAST (bedbkc23)=Indicator(1) /CONTRAST (bedbxc23)=Indicator(1) /CONTRAST (bedctc23)=Indicator(1) /CONTRAST (bedvc23)=Indicator(1) /CONTRAST (bedovc23)=Indicator(1) /CONTRAST (bedwlc23)=Indicator(1) /CONTRAST (bedwc23)=Indicator(1) /CONTRAST (bedlc23)=Indicator(1) /CONTRAST (beddc23)=Indicator(1) /CONTRAST (bclc24)=Indicator(1) /CONTRAST (bclm24w)=Indicator(1) /CONTRAST (bclm24o)=Indicator(1) /CONTRAST (itltc25)=Indicator(1) /CONTRAST (itlwdirt)=Indicator(1) /CONTRAST (itltwsdt)=Indicator(1) /CONTRAST (itltwcom)=Indicator(1) /CONTRAST (sdutc26)=Indicator(1) /CONTRAST (noctc)=Indicator(1) /CONTRAST (htctc28)=Indicator(1) /CONTRAST (opet29)=Indicator(1) /CONTRAST (opetd29c)=Indicator(1) /CONTRAST (opetabc)=Indicator(1) /CONTRAST (opetb29c)=Indicator(1) /CONTRAST

(opetf29c)=Indicator(1) /CONTRAST (opetgpc)=Indicator(1) /CONTRAST (opeteqc)=Indicator(1) /CONTRAST (boct30)=Indicator(1) /CONTRAST (wgtc32)=Indicator(1) /CONTRAST (bhv33c)=Indicator(1) /CONTRAST (fgtc34c)=Indicator(1) /CONTRAST (muec35)=Indicator(1) /CONTRAST (afrc35)=Indicator(1) /CONTRAST (herc35)=Indicator(1) /CONTRAST (pesc35)=Indicator(1) /CONTRAST (fugc35)=Indicator(1) /CONTRAST (snilc35)=Indicator(1) /CONTRAST (pstc35)=Indicator(1) /CONTRAST (othc35)=Indicator(1) /CONTRAST (flyc36)=Indicator(1) /CONTRAST (ant36)=Indicator(1) /CONTRAST (flec36)=Indicator(1) /CONTRAST (pfr36)=Indicator(1) /CONTRAST (pch36)=Indicator(1) /CONTRAST (pst36)=Indicator(1) /CONTRAST (oth36)=Indicator(1) /CONTRAST (nibpc37)=Indicator(1) /CONTRAST (hovc38)=Indicator(1) /CONTRAST (smkc39)=Indicator(1) /CONTRAST (crtc40)=Indicator(1) /CONTRAST (poisc41)=Indicator(1) /CONTRAST (medc42)=Indicator(1) /CONTRAST (flecat)=Indicator(1) /CONTRAST (flebed)=Indicator(1) /CONTRAST (flec43c)=Indicator(1) /CONTRAST (flesc43)=Indicator(1) /CONTRAST (igrc43)=Indicator(1) /CONTRAST (afrc44)=Indicator(1) /CONTRAST (farm45)=Indicator(1) /CONTRAST (afrc45)=Indicator(1) /CONTRAST (mrlkc46)=Indicator(1)

/METHOD=FSTEP(COND) lawn19 gard19 orch19 past19 crfld19 park19 plygr19 othoi toutc12 loutc21 slep bedbc23 bedsc23 bedcc23 bedbbc23 bedfc23 bedbkc23 bedbxc23 bedctc23 bedvc23 bedovc23 bedwlc23 bedwc23 bedlc23 beddc23 bclc24 bclm24w bclm24o itltc25 itlwdirt itltwsdt itltwcom sdutc26 noctc htctc28 opet29 opetd29c opetabc opetb29c opetf29c opetgpc opeteqc boct30 wgtc32 bhv33c fgtc34c muec35 afrc35 herc35 pesc35 fugc35 snilc35 pstc35 othc35 flyc36 ant36 flec36 pfr36 pch36 pst36 oth36 nibpc37 hovc38 smkc39 crtc40 poisc41 medc42 flecat flebed flec43c flesc43 igrc43 afrc44 farm45 afrc45 mrlkc46

/PRINT=CI(95)

/CRITERIA=PIN(.05) POUT(.10) ITERATE(20) .

Cat diet and feeding practices

COXREG

srvtypec /STATUS=srvtyper(1) /STRATA=triadid

/CONTRAST (fho49)=Indicator(1) /CONTRAST (fdf50c)=Indicator(1)
/CONTRAST (m51)=Indicator(1) /CONTRAST (alt52)=Indicator(1)
/CONTRAST (dr52)=Indicator(1) /CONTRAST (drp52c2)=Indicator(1)
/CONTRAST (drfv52c)=Indicator(1) /CONTRAST (cn52)=Indicator(1)
/CONTRAST (cnp52c2)=Indicator(1) /CONTRAST
(cnfv52c)=Indicator(1) /CONTRAST (pr52)=Indicator(1) /CONTRAST
(pt52)=Indicator(1) /CONTRAST (rm52)=Indicator(1) /CONTRAST
(dp52)=Indicator(1) /CONTRAST (cm52)=Indicator(1) /CONTRAST
(vftveg)=Indicator(1) /CONTRAST (vftjunk)=Indicator(1) /CONTRAST
(ct52)=Indicator(1) /CONTRAST (ad53)=Indicator(1) /CONTRAST
(fot54c)=Indicator(1) /CONTRAST (liq55)=Indicator(1) /CONTRAST
(liqq56c)=Indicator(1) /CONTRAST (trw57)=Indicator(1) /CONTRAST
(tab57)=Indicator(1) /CONTRAST (tcs57)=Indicator(1) /CONTRAST
(tfl57)=Indicator(1) /CONTRAST (sk57)=Indicator(1) /CONTRAST
(bh57)=Indicator(1) /CONTRAST (sh57)=Indicator(1) /CONTRAST
(tt57)=Indicator(1) /CONTRAST (fps57)=Indicator(1) /CONTRAST
(pdl57c)=Indicator(1) /CONTRAST (gp57)=Indicator(1) /CONTRAST
(ssp57c)=Indicator(1) /CONTRAST (at57)=Indicator(1) /CONTRAST
(oth57c)=Indicator(1) /CONTRAST (mk58c)=Indicator(1) /CONTRAST
(dp59)=Indicator(1) /CONTRAST (de59)=Indicator(1) /CONTRAST
(ds59)=Indicator(1) /CONTRAST (dc59)=Indicator(1) /CONTRAST
(doth59c)=Indicator(1) /CONTRAST (tdr60c15)=Indicator(1)
/CONTRAST (tndrc15)=Indicator(1) /CONTRAST (dclf62r)=Indicator(1)
/CONTRAST (mic63)=Indicator(1) /CONTRAST (micf63c)=Indicator(1)

/METHOD=FSTEP(COND) fho49 fdf50c m51 alt52 dr52 drp52c2 drfv52c cn52 cnp52c2 cnfv52c pr52 pt52 rm52 dp52 cm52 vftveg vftjunk ct52 ad53 fot54c liq55 liqq56c trw57 tab57 tcs57 tfl57 sk57 bh57 sh57 tt57 fps57 pdl57c gp57 ssp57c at57 oth57c mk58c dp59 de59 ds59 dc59 doth59c tdr60c15 tndrc15 dclf62r mic63 micf63c

/PRINT=CI(95)

/CRITERIA=PIN(.05) POUT(.10) ITERATE(20) .

Second part of this analysis - pre-final model for the four risk factor groupings (mentioned above)

COXREG

srvtypec /STATUS=srvtyper(1) /STRATA=triadid

/CONTRAST (brdslsio)=Indicator(1) /CONTRAST (dsexya)=Indicator(1) /CONTRAST (cfbac14)=Indicator(1) /CONTRAST (drhc14)=Indicator(1) /CONTRAST (noctc)=Indicator(1) /CONTRAST (muec35)=Indicator(1) /CONTRAST (flyc36)=Indicator(1) /CONTRAST (cnp52c2)=Indicator(1) /CONTRAST (cnfv52c)=Indicator(1) /CONTRAST (dp52)=Indicator(1) /CONTRAST (vftveg)=Indicator(1) /CONTRAST (liqq56c)=Indicator(1) /CONTRAST (pdl57c)=Indicator(1) /CONTRAST (mk58c)=Indicator(1)

/METHOD=FSTEP(COND) brdslsio dsexya cfbac14 drhc14 noctc muec35 flyc36 cnp52c2 cnfv52c dp52 vftveg liqq56c pdl57c mk58c

/PRINT=CI(95)

/CRITERIA=PIN(.05) POUT(.10) ITERATE(20) .

Third part of this analysis - final model for the four risk factor groupings with interactions

```
COXREG
  srvtypec /STATUS=srvtyper(1) /STRATA=triadid
  /CONTRAST (drhc14)=Indicator(1)  /CONTRAST (noctc)=Indicator(1)
/CONTRAST (muec35)=Indicator(1)  /CONTRAST (flyc36)=Indicator(1)
/CONTRAST (cnfv52c)=Indicator(1) /CONTRAST (pdl57c)=Indicator(1)
  /METHOD=ENTER drhc14 noctc muec35 flyc36 cnfv52c pdl57c
  /METHOD=FSTEP(COND)          drhc14*noctc          drhc14*muec35
drhc14*flyc36      cnfv52c*drhc14      drhc14*pdl57c      muec35*flyc36
cnfv52c*noctc muec35*pdl57c
  /PRINT=CI(95)
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20) .
```

Cox regression (proportional hazards model) for case-random status for comparing the survival and hazard curves for the risk factors present in the final case-random model

```
COXREG
  age5 /STATUS=srvtype(1)
  /PATTERN BY dsexya
  /CONTRAST (sex)=Indicator(1)  /CONTRAST (brdslsio)=Indicator(1)
/CONTRAST (dsexya)=Indicator(1)  /CONTRAST (denc14)=Indicator(1)
/CONTRAST (bedfc23)=Indicator(1)  /CONTRAST (flebed)=Indicator(1)
/CONTRAST (cnp52c2)=Indicator(1)
  /METHOD=FSTEP(LR) sex brdslsio dsexya denc14 bedfc23 flebed
cnp52c2
  /PLOT SURVIVAL HAZARD
  /PRINT=CI(95) BASELINE
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20) .
```

Kaplan-Meier survival analysis for cases used for comparing the different treatments on survival time (months) of hyperthyroid cats since the diagnosis up to the final date of the study

```
KM
 dgage16  BY tnon18  /STATUS=dead5(1)
 /PRINT TABLE MEAN
 /PLOT SURVIVAL HAZARD
 /TEST LOGRANK
 /COMPARE OVERALL POOLED .
```