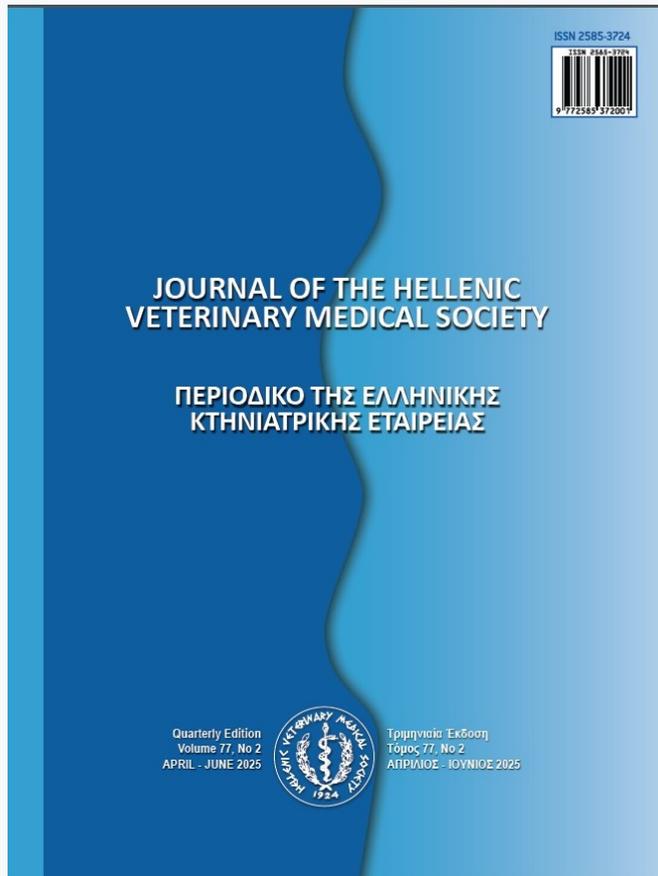


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Prevalence of feline hyperthyroidism in Greece

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ABSTRACT: Hyperthyroidism is the most common endocrine disorder of cats and usually occurs in middle-aged to older animals. The primary aim of this study was to determine the prevalence of feline hyperthyroidism in Greece, and a secondary aim was to compare the clinical signs, haematological and serum biochemical findings between hyperthyroid and non-hyperthyroid cats. A total of 80 cats, six years of age or older, were prospectively enrolled. The diagnosis of hyperthyroidism was based on serum concentrations of total thyroxine (measured by a solid-phase chemiluminescent competitive assay) and free thyroxine (measured by an equilibrium dialysis radioimmunoassay). Hyperthyroidism was diagnosed in 4/80 (5%) of the cats. Clinical and laboratory findings that were significantly more common in hyperthyroid compared to the non-hyperthyroid cats included a palpable thyroid nodule, depression-lethargy, dyspnoea, and decreased blood urea nitrogen concentration. Feline hyperthyroidism appears to be relatively common in Greece and routine measurement of thyroid hormone concentration should be considered in all middle-aged and older cats.

Keyword: Cattle; biochemistry; clinical signs; haematology; hormone; epidemiology; thyroid.

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INTRODUCTION

Hyperthyroidism is a multi-systemic disorder resulting from excessive circulating concentrations of thyroid hormones, namely thyroxine (T_4) and triiodothyronine (T_3). It occurs mainly in middle-aged and older cats, with a reported age range of 2-25 years and a median age at the time of diagnosis of 11.5-15.1 years (Milner et al., 2006; Williams et al., 2010; Peterson, 2013a; Peterson and Broome, 2015; Puig et al., 2015; Grossi et al., 2019). Benign adenoma or adenomatous hyperplasia of the thyroid are the most common causes of the disease, accounting for 97-99% of the cases (Turrel et al., 1988; Peterson et al., 1994; Naan et al., 2006).

A wide range of clinical signs is seen in hyperthyroid cats, including weight loss, increased appetite, polyuria/polydipsia, vomiting, diarrhoea, hyperactivity, tachypnoea, tachycardia, and a palpable goitre (Broussard et al., 1995; Frenais et al., 2009; Nussbaum et al., 2015; Grossi et al., 2019). Less commonly, cats present the apathetic form of the disease, that is characterized by lethargy, weakness and decreased appetite, and is usually accompanied by concurrent severe systemic diseases (Thoday and Mooney, 1992; Bucknell, 2000; Frenais et al., 2009; Nussbaum et al., 2015; Grossi et al., 2019).

The most common haematologic and serum biochemistry abnormalities associated with feline hyperthyroidism include anaemia, leucocytosis, increased activity of alanine aminotransferase (ALT) and alkaline phosphatase (ALP), decreased calcium, increased inorganic phosphorous and decreased potassium concentrations (Peterson and Ferguson, 1983; Thoday and Mooney, 1992; Broussard et al., 1995; Milner et al., 2006; Naan et al., 2006; Frenais et al., 2009; Williams et al., 2013; Hiebert et al., 2020).

Hyperthyroidism is the most common endocrinopathy of cats, with a prevalence of 1-4% in the general feline population and higher than 6% in cats older than 9 years (Milner et al., 2006; Trepanier, 2006; Wakeling et al., 2011). Since its initial description (Peterson et al., 1979) the prevalence (or the frequency of diagnosis) of the disease has been steadily increasing worldwide (McLean et al., 2017), and there is strong evidence that it differs among different geographical areas (Scarlett et al., 1988). Depending on the selection criteria (mainly the age) of the cats enrolled in each study, reported prevalence rates vary widely, from 0.2% to 21.1% in Europe,

from 0.1 to 2% in North America, and from 3.9% to 8.9% in Asia (Edinboro et al., 2004; De Wet et al., 2009; Wakeling et al., 2011; McLean et al., 2017). The prevalence of feline hyperthyroidism in Greece remains unknown.

The primary aim of this study was to determine the prevalence of feline hyperthyroidism in Greece. A secondary aim was to compare the clinical signs, haematological and serum biochemical findings between hyperthyroid and non-hyperthyroid cats.

MATERIALS AND METHODS

Ethics approval

The study protocol was reviewed and approved by the Animal Ethics Committee of the Faculty of Veterinary Science, University of Thessaly (licence No: 13/16-6-15). Handling of the cats was in compliance with the European Communities Council Directive 2010/63/EU and State laws.

Study population

Cats from four different areas of Greece (Attica, Thessaly, Crete, and Macedonia) were prospectively enrolled, by three clinicians (KCGK, PGX, MEM), between March 2014 and July 2016. Client-owned cats, cats living in catteries, and stray cats that were presented by their owners or by volunteer cat-rescue groups for wellness examination, vaccination, neutering and/or medical treatment, were enrolled if they were at least 6 years-old [the age of stray cats was estimated based on body size, dentition and other physical characteristics (Hale, 2005; Bellows et al., 2016; DiGangi et al., 2020)] and an informed consent form was signed by the owner or the rescuer. The only exclusion criterion was previous diagnosis and/or treatment of hyperthyroidism.

Signalment and historical data were collected using a standardized questionnaire followed by a thorough physical examination and blood sampling.

Sample collection and laboratory analyses

A total of 5 ml of blood was collected from the jugular vein. One ml was immediately transferred into EDTA-anticoagulated tube and was used for haematology, whereas the remaining blood was centrifuged, and serum was separated and stored at -80°C until further use.

Serum total T_4 (tT_4) concentration was measured by a solid-phase chemiluminescent competitive assay (Immulite 2000 Canine Total T_4 , Siemens Healthcare Diagnostics, Deerfield, IL) with a refer-

ence interval of 0.78-3.82 µg/dl. Serum free T₄ (fT₄) concentration was measured by an equilibrium dialysis radioimmunoassay [Free T₄ (by ED), Antech Diagnostics, Irvine, CA] with a reference interval of 0.7-2.6 ng/dl. A cat was considered hyperthyroid if: a) serum tT₄ concentration was above the upper limit of the reference interval or b) tT₄ concentration was in the upper half of the reference interval (i.e. >2.3 µg/dl), fT₄ concentration was increased, and there was a palpable goitre (Peterson and Broome, 2015).

Haematology was performed, within 12 h of blood collection, with one of three different analysers (Sysmex pochH-100i, ADVIA 2120i Siemens, ADVIA 120 Siemens), depending on sampling area. The results for each haematology parameter were classified as increased, normal, or decreased, based on the reference intervals of the analyser.

Biochemical parameters were measured using an automated analyser (Roche/Hitachi MODULAR ANALYTICS D 2400 module, Roche Diagnostics, Switzerland, CH).

Statistical analysis

Categorical data from signalment, history, clinical examination, haematology, and serum biochemistry were compared between hyperthyroid and euthyroid cats using either Pearson's χ^2 or Fisher's exact test. The distribution of continuous variables was tested using a Kolmogorov-Smirnov test. Normally distributed variables are presented as mean \pm standard deviation (SD) and were compared between hyperthyroid and euthyroid cats using independent samples *t*-tests. Non-normally distributed variables are presented as median and range and were compared between hyperthyroid and euthyroid cats using the Mann Whitney *U* test. Analyses were done using SPSS 23 for Windows (IBM Corp, Armonk, NY) and the level of significance was set at $P < 0.05$.

RESULTS

A total of 80 cats were included in the study; 70 (87.5%) of them were sick and 10 (12.5%) were clinically healthy.

A total of 4/80 (5%) cats were diagnosed with hyperthyroidism. All of them were sick, and 3/4 (75%) had increased tT₄ (5.39 µg/dl, 6.32 µg/dl, and 8.49 µg/dl) and fT₄ (4.73 ng/dl, 5.56 ng/dl and >6 ng/dl, respectively) concentrations, and a palpable goitre. The fourth cat had a tT₄ concentration in the upper half of the reference interval (2.35 µg/dl), an increased fT₄ concentration (3.26 ng/dl), a palpable

goitre, and presented the apathetic form of hyperthyroidism. This cat also had clinical signs of upper respiratory infection, anaemia, decreased serum albumin and increased serum globulin concentration, it was seropositive for feline immunodeficiency virus, *Toxoplasma gondii*, and *Bartonella* spp. and it was blood PCR-positive for *Mycoplasma* spp.

The remaining 76/80 (95%) euthyroid cats were sick (66/76-86.8%) or clinically healthy (10/76-13.2%), and their tT₄ (0.63-3.27 µg/dl) and fT₄ (0.4-2.29 ng/dl) concentrations were below the upper limit of the reference interval. Also, 1/76 (1.3%) had a palpable goitre.

The age of the four hyperthyroid cats ranged from 6 to 13 years (median: 9 years). Two (50%) of them were neutered males, and 2 (50%) spayed females. All were domestic shorthair, and their body weight was 2.75 ± 0.35 kg. The age of the 76 euthyroid cats ranged from 6 to 17 years (median: 8 years). Thirty-nine (51.3%) of them were males (29 castrated) and 37 (48.7%) females (26 spayed). Four (5.3%) were purebred, 70 (92.1%) domestic shorthairs, and the breed of 2 (2.6%) cats was not recorded. Their body weight was 4.35 ± 1.41 kg. There was no significant difference in the age ($P = 0.975$), sex ($P = 1$) or body weight ($P = 0.115$) between hyperthyroid and euthyroid cats.

The frequency of clinical manifestations, based on history and clinical examination (Table 1), and of haematology and serum biochemistry abnormalities (Table 2) were compared between hyperthyroid and euthyroid cats. The frequency of palpable goitre, depression-lethargy and dyspnoea was significantly higher in hyperthyroid compared to euthyroid cats (Table 1). Also, decreased blood urea nitrogen (BUN) concentration was more common in hyperthyroid cats (Table 2).

DISCUSSION

This is the first study reporting the prevalence of feline hyperthyroidism in Greece. Hyperthyroidism was diagnosed in 5% of cats older than 6 years (6 to 17 years). Palpable goitre, depression-lethargy, dyspnoea, and decreased BUN concentration were more common in hyperthyroid compared to euthyroid cats.

The diagnostic sensitivity of serum tT₄ concentrations for feline hyperthyroidism ranges between 90% and 95.2% but can be as low as 65% in the early stages of the disease and in cats with concurrent systemic illnesses (Milner et al., 2006; Peterson, 2013b; Peterson and Broome, 2015; Peterson et al.,

Table 1. Comparison of the frequency of clinical manifestations, based on history and clinical examination, between four hyperthyroid and 76 euthyroid cats (asterisks indicate variables that differ significantly between the two groups of cats)

Clinical sign	Missing data	Hyperthyroid (%)	Euthyroid (%)	P value
Weight loss	9	2/3 (66.7%)	15/68 (22.1%)	0.14
Anorexia (previous 3 weeks)	6	2/4 (50%)	22/70 (31.4%)	0.591
Increased appetite	9	0/4 (0%)	2/67 (3%)	1
Depression-lethargy	0	3/4 (75%)	15/76 (19.7%)	0.034*
Weakness	0	1/4 (25%)	3/76 (3.9%)	0.189
Dehydration	0	2/4 (50%)	15/76 (19.7%)	0.197
Fever	0	0/4 (0%)	4/76 (5.3%)	1
Lymphadenomegaly	0	0/4 (0%)	7/76 (9.2%)	1
Abnormal lung sounds	0	0/4 (0%)	3/76 (3.9%)	1
Cough	0	1/4 (25%)	0/76 (0%)	0.05
Dyspnoea	0	2/4 (50%)	2/76 (2.6%)	0.011*
Ocular signs	0	1/4 (25%)	12/76 (15.8%)	0.515
Gingivostomatitis	0	2/4 (50%)	22/76 (28.9%)	0.579
Vomiting	6	0/3 (0%)	8/71 (11.3%)	1
Diarrhoea	6	0/3 (0%)	4/71 (5.6%)	1
Constipation	8	0/3 (0%)	1/69 (1.4%)	1
Abdominal pain	0	0/4 (0%)	2/76 (2.6%)	1
Abdominal mass	0	0/4 (0%)	2/76 (2.6%)	1
Abdominal effusion	0	0/4 (0%)	2/76 (2.6%)	1
Hepatomegaly	0	0/4 (0%)	1/76 (1.3%)	1
Splenomegaly	0	1/4 (25%)	0/76 (0%)	0.05
Increased kidney size	0	1/4 (25%)	2/76 (2.6%)	0.144
Non-smooth kidney surface	0	0/4 (0%)	2/76 (2.6%)	1
Polyuria-polydipsia	10	0/3 (0%)	6/67 (9%)	1
Dysuria	7	0/3 (0%)	3/70 (4.3%)	1
Skin lesions and/or pruritus	0	2/4 (50%)	24/76 (31.6%)	0.592
Neurological signs	0	0/4 (0%)	2/76 (2.6%)	1
Musculoskeletal signs	0	0/4 (0%)	4/76 (5.3%)	1
Palpable goitre	0	3/4 (75%)	1/76 (1.3%)	<0.001*
At least one clinical sign/symptom	0	4/4 (100%)	66/76 (86.8%)	1
At least one clinical sign/symptom compatible with hyperthyroidism	0	4/4 (100%)	42/76 (55.3%)	0.133

2015). However, the diagnostic sensitivity of fT_4 in hyperthyroid cats with tT_4 concentration within reference interval is approximately 95% (Peterson, 2006). Because all 76 cats that were considered euthyroid had tT_4 and fT_4 concentrations within reference intervals, the possibility of having misclassified some hyperthyroid cats as euthyroid is small, although it cannot be totally excluded.

The diagnostic specificity of serum tT_4 concentration is very high, typically varying between 98% and 100% (Peterson, 2006, 2013b; Peterson and Broome, 2015). Also, the diagnostic specificity of increased fT_4 in cats with high-normal tT_4 and a palpable goitre is high (Peterson, 2013b) and for this reason the single cat with normal tT_4 and increased fT_4 was considered hyperthyroid. The authors acknowledge that

Table 2. Comparison of the frequency of haematology and serum biochemistry abnormalities between four hyperthyroid and 76 euthyroid cats (asterisks indicate variables that differ significantly between the two groups of cats)

Variable	Categories	Missing data	Hyperthyroid (%)	Euthyroid (%)	<i>P</i> value
HCT	Decreased	26	1/4 (25%)	16/50 (32%)	1
White blood cells	Decreased	26	0/4 (0%)	7/50 (14%)	1
	Increased		1/4 (25%)	11/50 (22%)	
Neutrophils	Decreased	26	1/4 (25%)	6/50 (12%)	0.306
	Increased		1/4 (25%)	7/50 (14%)	
Lymphocytes	Decreased	26	1/4 (25%)	14/50 (28%)	1
Monocytes	Increased	26	0/4 (0%)	1/50 (2%)	1
Eosinophils	Decreased	26	0/4 (0%)	3/50 (6%)	1
	Increased		1/4 (25%)	9/50 (18%)	
Platelets	Decreased	26	1/4 (25%)	13/50 (26%)	1
	Increased		0/4 (0%)	4/50 (8%)	
Total proteins	Decreased	2	0/3 (0%)	2/75 (2.7%)	0.28
	Increased		1/3 (33.3%)	5/75 (6.7%)	
Albumins	Decreased	0	4/4 (100%)	63/76 (82.9%)	1
Globulins	Decreased	2	0/3 (0%)	1/75 (1.3%)	0.612
	Increased		2/3 (66.7%)	34/75 (45.3%)	
Urea nitrogen	Decreased	3	2/3 (66.7%)	7/74 (9.5%)	0.017*
	Increased		1/3 (33.3%)	10/74 (13.5%)	
Creatinine	Increased	2	1/4 (25%)	7/74 (9.5%)	0.346
Glucose	Increased	2	2/3 (66.7%)	41/75 (54.7%)	1
Cholesterol	Increased	3	1/3 (33.3%)	1/74 (1.4%)	0.111
Triglycerides	Decreased	2	0/3 (0%)	3/75 (4%)	1
	Increased		0/3 (0%)	4/75 (5.3%)	
Total bilirubin	Increased	1	1/4 (25%)	3/75 (4%)	0.191
ALP	Increased	0	1/4 (25%)	3/76 (3.9%)	0.189
ALT	Increased	4	0/4 (0%)	0/72 (0%)	
AST	Increased	1	1/4 (25%)	8/75 (10.7%)	0.39
γ-GT	Increased	5	0/3 (0%)	1/72 (1.4%)	1
Calcium	Decreased	2	0/4 (0%)	6/74 (8.1%)	1
	Increased		0/4 (0%)	1/74 (1.4%)	
Inorganic phosphorus	Increased	3	2/4 (50%)	8/73 (11%)	0.08
Potassium	Decreased	1	0/3 (0%)	1/76 (1.3%)	1
	Increased		0/3 (0%)	10/76 (13.2%)	
Sodium	Decreased	1	1/3 (33.3%)	15/76 (19.7%)	0.63
	Increased		0/3 (0%)	6/76 (7.9%)	
Chloride	Decreased	1	2/3 (66.7%)	27/76 (35.5%)	0.569
	Increased		0/3 (0%)	1/76 (1.3%)	

ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; γ-GT: gamma-glutamyl transpeptidase; HCT: haematocrit

it would have been ideal, for this particular cat, to repeat the measurement of thyroid hormones and/or to perform alternative diagnostic tests (e.g., scintigraphy, triiodothyronine suppression test) to definitively confirm the diagnosis. Unfortunately, this was not feasible due to the design of the study and the long period between enrolment of the cat and measurement of thyroid hormone concentrations.

Our results are comparable with those of most previous studies, where the prevalence of hyperthyroidism in middle-aged and older cats was found to vary between 2% and 12% (Kraft and Buchler, 1999; Wakeling and Melian, 2005; Sassnau, 2006; Wakeling et al., 2011; Dias Neves and Horspool, 2014; O'Neill et al., 2014; Stephens et al., 2014; Köhler et al., 2016). However, higher prevalence has also been reported, such as 20% in Poland (Gójska Zygner et al., 2014) and 21.1% in Ireland (Bree et al., 2018), and the prevalence of the disease is increasing worldwide, thus necessitating routine clinical (palpation for goitre) and laboratory (measurement of tT_4 concentration) testing for hyperthyroidism in all middle-aged and older cats.

The palpation of one or two enlarged thyroid nodules is generally considered sensitive for the diagnosis of hyperthyroidism (Peterson, 2013b). However, the diagnostic sensitivity of this examination varies from 71% to 96% (Paepe et al., 2008; Hibbert et al., 2009), depending on the palpation technique (classical and/or "Norsworthy"), the conformation of the neck of the cat, the size of the thyroid nodule (very small nodules may not be palpable and large nodules may descend and enter into the thoracic cavity) and the source of excessive thyroid hormone production (thyroid gland or ectopic thyroid tissue) (Broome, 2006; Peterson, 2013b; Peterson and Broome, 2015). The above may explain why goitre was not palpable in 1/4 hyperthyroid cats in the present study. In contrast to the sensitivity, the diagnostic specificity of this examination is considered poor because a palpable nodule may represent non-functional thyroid tissue (e.g. thyroid cyst, thyroiditis), early stages of functional thyroid tissue that does not produce enough hormones to result in hyperthyroidism, enlarged parathyroid glands and also because of misinterpretation of the finding by inexperienced examiners (Peterson, 2006; Paepe et al., 2008; Wakeling et al., 2011; Peterson, 2013b). Even when 30 hyperthyroid and euthyroid cats were examined, independently, by three experienced clinicians, that were blinded to the thyroid status of each cat, the specificity of palpation ranged from 63.3% to

66.7% (Paepe et al., 2008). In light of these data, the high specificity (98.7%) of thyroid palpation (Table 1) was a surprising finding of our study. However, this might be explained by the small number of hyperthyroid cats included. Furthermore, we confirmed previous statements that cats with palpable nodules in the ventral neck region are significantly more likely to be hyperthyroid compared to cats without (McLean et al., 2017; Bree et al., 2018).

The majority (3/4) of the hyperthyroid cats of our study presented the apathetic form of the disease with depression or lethargy instead of hyperactivity and aggressiveness (Thoday and Mooney, 1992; Bucknell, 2000). Most likely the apathetic form occurred due to comorbidities or due to severe complications of hyperthyroidism, like heart failure that could also explain the increased prevalence of dyspnoea. The unexpected high prevalence of apathetic hyperthyroidism was probably the result of the inclusion and exclusion criteria of the study: cats with the classical clinical signs of the disease (hyperactivity, aggression, etc.) are more likely to be tested and diagnosed with hyperthyroidism by their regular veterinarians and such a previous diagnosis was an exclusion criterion for our study.

In the current study, dyspnoea was more common in hyperthyroid compared to euthyroid cats. The same has been reported in a prospective study of 508 cats from Ireland (Gallagher and Mooney, 2013), whereas the prevalence of dyspnoea among hyperthyroid cats has been reported to range between 11% and 32% (Peterson and Ferguson, 1983; Grossi et al., 2019). Dyspnoea in hyperthyroid cats has been attributed to congestive heart failure, heat intolerance, and/or various respiratory comorbidities (Syme, 2007; Sangster et al., 2013).

Finally, the increased frequency of low serum BUN concentrations in hyperthyroid compared to euthyroid cats, although not previously reported, can be explained by the increased glomerular filtration rate, which is present in hyperthyroidism (Syme, 2007).

Limitations of this study include the relatively small number of cats tested, and the inability to definitively confirm hyperthyroidism in the cat with normal tT_4 and high fT_4 concentration. Another limitation is the fact that the age of stray cats was approximate and was estimated based on their body size, dentition (including dental attrition), and other physical characteristics. Also, haematology was performed in different laboratories, using different analysers, depending on the region of sampling. For

this reason, in the statistical analysis we considered the results of haematology as categorical variables (normal, increased or decreased) that were defined based on the reference intervals of each analyser. Future prospective studies, including a larger number of cats and using additional testing to confirm the diagnosis in equivocal cases, are warranted to determine the risk factors for the appearance of the disease in Greece and to investigate further its clinical and clinicopathologic manifestations.

CONCLUSION

The results of this study indicate that hyperthyroidism is relatively common in cats older than 6 years in Greece. The disease must be considered in any middle-aged or older cat, especially if it has a palpable goitre, depression-lethargy, dyspnoea and/or decreased BUN concentration.

CONFLICT OF INTEREST STATEMENT

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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