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When typical clinical signs are not enough: overdiagnosis of canine hypothyroidism in Costa Rica

Cuando los signos clínicos típicos no bastan: sobrediagnóstico de hipotiroidismo canino en Costa Rica

Quando os sinais clínicos típicos não são suficientes, sobrediagnóstico de hipotireoidismo canino na Costa Rica

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Abstract
Canine hypothyroidism is the most common endocrine disease in dogs and is often overdiagnosed. The purpose of this study is to analyze the importance of testing TSH together with free Thyroxine (fT4) to differentiate between true hypothyroid cases and suspected cases with clinical signs suggestive of hypothyroidism and identify if there is any level of canine hypothyroidism overdiagnosis. There were 48 individuals in the control group (clinically healthy animals) (CT), 40 with clinical signs but with no laboratory confirmation (SHT), and 41 with laboratory confirmation (HT). Significant differences were obtained between the HT group and the C and SHT groups in both hormone values, but not between the C and SHT groups. A diagnosis solely based on clinical signs rather than laboratory diagnostic methods, the use of a single hormone test, the method used for measuring hormones, and any possible interference can cause false positives in the diagnosis of canine hypothyroidism. Therefore, all these factors must be considered when making a diagnosis, leading the clinician to use a multiple-approach diagnosis rather than a single-approach diagnosis.

Keywords: Hypothyroidism, TSH, fT4, overdiagnosis, dogs, thyroid gland, hormone assays, laboratory.

Resumen
El hipotiroidismo canino es la enfermedad endocrina más común en perros y está sobre diagnosticada. El propósito de este estudio es analizar la importancia de la medición de la TSH en conjunto con la Tiroxina libre (fT4) con el objetivo de diferenciar entre casos de hipotiroidismo verdadero y casos sospechosos con signos clínicos sugestivos de hipotiroidismo e identificar si existe algún nivel de sobrediagnóstico de hipotiroidismo canino. Se obtuvieron un total de 48 individuos en el grupo control (animales clínicamente sanos) (CT), 40 individuos con signos clínicos, pero sin confirmación de laboratorio (SHT) y 41 individuos con confirmación de laboratorio (HT). Se obtuvieron diferencias significativas entre el grupo HT y los grupos C y SHT en ambos valores hormonales, pero no hubo diferencias significativas entre los grupos C y SHT. El diagnóstico basado únicamente en signos clínicos, no utilizar

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métodos de diagnóstico laboratorial, el uso de una sola prueba hormonal, el método de medición hormonal utilizado y las posibles interferencias, pueden causar falsos positivos en el diagnóstico de hipotiroidismo canino. Por lo tanto, todos estos factores deben ser considerados cuando se realiza el diagnóstico, utilizando un enfoque diagnóstico múltiple y no un solo elemento diagnóstico.

Palabras clave: Hipotiroidismo, TSH, fT4, sobrediagnóstico, perros, glándula tiroides, pruebas hormonales, laboratorio.

Resumo
O hipotireoidismo canino é a doença endócrina mais comum em cães e está sobre diagnosticada, o propósito deste estudo é analisar a importância da medição do TSH em conjunto com a Tiroxina livre (fT4) com o objetivo de diferenciar entre casos de hipotireoidismo verdadeiro e casos suspeitos com sinais clínicos sugestivos de hipotireoidismo e identificar se existe algum nível de sobrediagnóstico de hipotireoidismo canino. Foram obtidos um total de 48 indivíduos no grupo controle (animais clinicamente saudáveis) (CT), 40 indivíduos com sinais clínicos, mas sem confirmação laboratorial (SHT) e 41 indivíduos com confirmação laboratorial (HT). Foram obtidas diferenças significativas entre o grupo HT e os grupos C e SHT em ambos os valores hormonais, mas não houve diferenças significativas entre os grupos C e SHT. Um diagnóstico baseado exclusivamente em sinais clínicos, sem a utilização de métodos de diagnóstico laboratorial, apoiado no uso de um único teste hormonal, desconsiderando o método de medição hormonal e as possíveis interferências, pode levar a diagnósticos falsos positivos de hipotireoidismo canino. Portanto, é essencial considerar todos esses fatores ao realizar o diagnóstico, adotando uma abordagem diagnóstica múltipla em vez de se basear em um único elemento diagnóstico.

Palavras-chave: Hipotireoidismo, canino, TSH, fT4, sobrediagnóstico, glândula tireoide, análise hormonal, laboratório

Introduction

Canine hypothyroidism is the most common endocrine disease in dogs (*Canis lupus familiaris*) (Graham & Mooney, 2012). It may occur due to thyroid gland destruction, decreased stimulation by thyroid stimulating hormone (TSH), or failure in any of the steps of thyroid hormone synthesis (Scott-Moncrieff, 2015).

Hypothyroidism can be acquired or can be congenital. It is classified into primary (abnormalities in the thyroid gland), secondary (decreased TSH), and tertiary (thyrotropin-releasing hormone deficiency). The most common and best-described etiology of hypothyroidism is the primary form, which occurs in 95% of reported cases, and its main known causes are idiopathic thyroid atrophy and lymphocytic thyroiditis. Secondary hypothyroidism is rare and tertiary hypothyroidism is presumed to be extremely rare (Scott-Moncrieff, 2015).

Hypothyroidism affects mainly dogs between 4 and 10 years old, the average age being 7 years old (Dixon, 2013). The most predisposed breeds to the disease are the Doberman pinscher, Miniature Schnauzer, Golden Retriever, Cocker Spaniel, and Beagle (Dixon, 2013). Its prevalence among the canine population is not fully known, although studies report that it is between 0.2% and 0.8% (Mooney, 2011).

Due to the wide range of functions of thyroid hormones, their absence has nonspecific clinical signs in various systems and body tissues (Panciera et al., 2012). The most frequently reported clinical signs are dermatological (88% of cases), metabolic, and hematologic such as: symmetric bilateral alopecia, “rat tail”, obesity (49% of cases), weakness (12% of cases), lethargy (49% of cases), anemia, hyperlipidemia, myxedema, and cold intolerance. However, in some cases, literature also mentions alterations inherent to other systems

such as neurological, cardiovascular, and reproductive signs in which, even, low birth weight of the offspring and per partum mortality can be observed (Dixon, 2013; Panciera et al., 2012; Scott-Moncrieff, 2015).

The diagnosis of this disease is complex, not only because of its lack of specificity and great variation in the presentation of its signs but also because of the difficulty of interpreting the results of diagnostic tests. It is probably one of the most overdiagnosed veterinary diseases, although, under certain circumstances, it can also be underdiagnosed (Graham & Mooney, 2012).

The purpose of this study is to analyze the importance of measuring TSH together with free Thyroxine (fT4) to differentiate between true hypothyroid cases and suspected cases with clinical signs suggestive of hypothyroidism and to identify if there is any level of canine hypothyroidism overdiagnosis made by Costa Rican veterinary clinicians and determine probable causes of this problem.

Materials and methods

Study population

Purebred dogs and mixed-breed dogs in the Greater Metropolitan Area of Costa Rica (which includes the provinces of San José, Alajuela, Heredia, and Cartago) were selected for the study. Samples were submitted by veterinary clinics with the owners' authorization, who voluntarily offered their pets' participation in the study. There were 48 individuals in the control group (clinically healthy animals), 40 with clinical signs but no laboratory confirmation, and 41 with laboratory confirmation. Their ages ranged between 1 and 10 years, and no distinction between sex or reproductive condition was made. Pregnant or lactating females were not admitted into the study. Males and females had to be free of any medication that could alter the values of thyroid hormones for at least 6 weeks prior to the time of sampling. Hypothyroidism was confirmed when TSH levels were higher than 0.5ng/mL.

Patient information and specifications for sampling methods and sample handling and submission

The veterinarian collaborating with the study filled out a questionnaire for each case of suspected or confirmed canine hypothyroidism. The questionnaire included the full medical history of subjects and information on their physical examination on the sampling day, which allowed us to meet the minimal requirements for the study and analyze any signs of hypothyroidism in any patient.

A second document was given to all participating clinicians indicating the requirements to participate, materials specifications, sampling methods, labeling, centrifugation procedures, sample handling and submission, contraindicated medications and adequate medication withdrawal time, and any possible interference with measurements in the sample.

Hormones assay

Specific Canine TSH and free T4 (fT4) were determined with the Siemens IMMULITE 1000 Immunoassay Analyzer following the specifications of use determined by the manufacturer.

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Canine TSH (canine thyrotropin, cTSH) was quantitatively measured by a solid phase, two-phase, two-site chemiluminescent immunometric assay (IMMULITE 1000 canine TSH kit) in serum.

Free thyroxine was measured by a solid-phase, enzyme-labeled chemiluminescent competitive immunoassay (IMMULITE 1000 Free T4) for the quantitative measurement of non-protein-bound thyroxine (free T4) levels in serum and heparinized plasma. Free T4 is a solid-phase, enzyme-labeled chemiluminescent competitive immunoassay. The reportable range is 0.3-6 ng/dL (3.9-77.2 pmol/L). Results below 0.3 ng/mL are reported as 0.3 ng/mL.

Statistical analysis

Differences between groups were tested using an ANOVA test, which was performed using IBM SPSS®v24 with a standard α level of 0.05.

Results

A total of 129 dogs were included in the questionnaire, with ages ranging from 1 to 15 years (mean 5.97 ± 2.98), including 48 individuals in the control group (clinically healthy animals), 40 with clinical signs but no laboratory confirmation, and 41 with laboratory confirmation (Table 1).

Table 1. TSH and fT4 values of each group, HT (hypothyroidism), TSH (thyroid stimulating hormone), fT4 (free thyroxine).

	Analyte (Units)	Mean ± SD	Range	95% CI
Control	TSH (ng/mL)	0.15 ± 0.11	0.03-0.49	0.12-0.18
	fT4 (ng/dL)	1.39 ± 0.66	0.56- 4.8	1.19-1.58
Suspected HT	TSH(ng/mL)	0.19 ± 0.12	0.03-0.47	0.15-0.23
	fT4(ng/dL)	1.18 ± 0.34	0.36-1.81	1.08-1.29
Confirmed HT	TSH (ng/mL)	2.18 ± 1.72	0.53-9.08	1.63-2.72
	fT4 (ng/dL)	0.43 ± 0.21	0.26-1.36	0.36-0.50

There were significant differences between the HT group and the C and SHT groups in both hormone values, but no significant differences between the C and SHT groups (TSH F = 59.50, $p<0.05$; fT4 F = 51.56, $p<0.05$). TSH was significantly higher in the HT groups, and fT4 was significantly lower (Fig. 1).

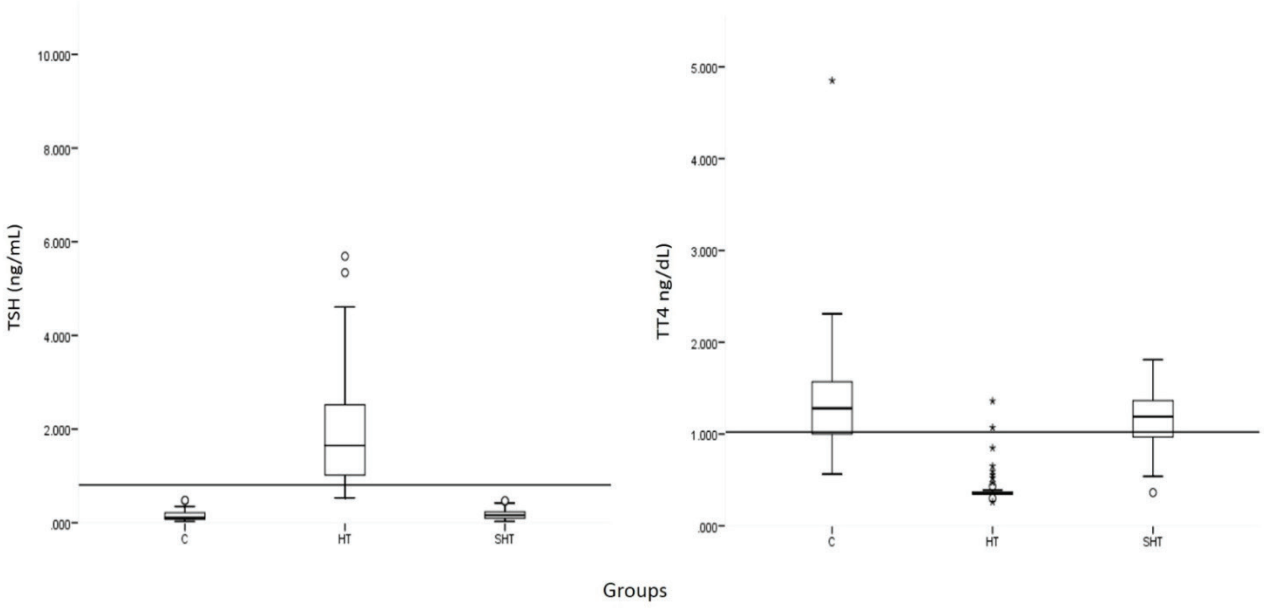

Figure 1. Box plot showing differences in TSH and fT4 values between groups. The transverse line indicates the mean value. C (control), HT (confirmed hypothyroidism), SHT (suspected hypothyroidism), TSH (thyroid stimulating hormone), fT4 (Free thyroxine).

Discussion

No significant differences were found in the hormonal values obtained between the C and SHT groups (Fig. 1). Clinical signs of hypothyroidism may be nonspecific and insidious in onset, which turns into a possible diagnosis within a wide range of different clinical scenarios (Scott-Moncrieff, 2007); thus, it is not possible to make the diagnosis of hypothyroidism based solely on clinical signs. The differences found in the values obtained for both hormones between the HT group and the C and SHT groups show the importance of hormonal tests to diagnose hypothyroidism, which relies largely on the assessment of basal thyroid hormone analyses, endogenous canine TSH, and the presence of TgAA (Mooney, 2011). Measuring thyroid hormone can be affected by age, race, medications (corticosteroids, antiepileptics, and sulfonamides), and nonthyroidal illness (Spence, 2022). It is important to mention that probable interferences were minimized to a feasible extent in the selection of patients for this study. If possible, interferences are not considered when performing hormonal tests because of the likelihood of false positive increments. For example, current disease can cause non-thyroidal illness (NTI) or "euthyroid sick syndrome" resulting in low TT4 concentration in the absence of hypothyroidism in approximately one-third of ill dogs (Mooney, 2011). Although serum fT4 concentrations are decreased to a lesser extent than total T4 concentrations (Kantrowitz et al., 2001; Peterson et al., 1997;) in the presence of severe systemic illness, fT4 concentrations can also be markedly decreased. In one study of 223 dogs with NTIS, approximately 31% of dogs had low total T4 concentrations and 22% had low fT4, whereas only 8% had high serum TSH concentrations (Kantrowitz et al. 2001). This is one of the most common causes of overdiagnosis of hypothyroidism (Spence, 2022).

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In this study, free T4 was used instead of total T4 because canine total T4 was unavailable in the country at that time. While the gold standard for free T4 measurement is equilibrium dialysis (Ferguson, 2007), the measurement of free T4 by chemiluminescence methods and equilibrium dialysis is most likely correlated in the absence of binding kinetics alterations (Schachter et al., 2004).

FT4 is slightly less sensitive than total T4 for diagnosing hypothyroidism (20% of dogs have values within the low-normal reference interval) (Dixon et al., 1999); however, fT4 is more specific because it is less affected by non-thyroidal illnesses (Spence, 2022). Caution should be exercised when fT4 measured by chemiluminescence methods alone is used to screen dogs for hypothyroidism. For example, 25–38% of TGA-positive hypothyroid dogs had fT4 results determined by chemiluminescence methods that did not support the diagnosis of hypothyroidism (Randolph et al., 2015).

In conclusion, a diagnosis solely based on clinical signs of hypothyroidism rather than laboratory diagnostic methods, the use of a single hormone test, the method used for measuring hormones, and any possible interference (i.e., drugs, non-thyroid diseases) can cause false positives in the diagnosis of canine hypothyroidism. Therefore, all these factors must be considered when the diagnosis is made, leading the clinician to use a multiple-approach diagnosis rather than a single-approach diagnosis.

Conflict of Interest: The authors declare no conflict of interest.

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