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Thyroid Testing in Sloughis

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Background: Thyroid hormone concentrations were found to be different in Greyhounds and Whippets compared with nonsight hound dogs.

Hypothesis: In Sloughis, thyroid hormone concentration is lower than in nonsight hounds and comparable to Greyhounds.

Animals: Fifty-one Sloughis with no evidence of disease and a mean age of 4 years (range, 1–12 years).

Methods: Thyroid profiles consisting of total thyroxine (tT4), free thyroxine (fT4), free thyroxine after equilibrium dialysis (fT4 after ED), canine thyroid stimulation hormone (cTSH), and thyroglobulin antibodies as well as CBC and serum biochemistry results of Sloughis were compared with those of normal dogs. In 8 Sloughis, TSH stimulation tests were performed.

Results: In Sloughis, tT4 concentrations and fT4 concentrations measured by chemiluminescence were lower than those of controls (1.13 ± 0.65 μg/dL compared with 2.9 ± 0.8 μg/dL, $P < .0001$ and 11 ± 4.3 pmol/L compared with 16.7 ± 5.2 pmol/L, $P < .0001$, respectively). Concentrations of fT4 after ED and TSH were increased in Sloughis, when compared with controls (41.3 ± 26.9 pmol/L compared with 20.98 ± 10.29 pmol/L, $P < .0001$ and 0.22 ± 0.15 pmol/L compared with 0.15 ± 0.13 pmol/L, $P = .0138$, respectively). T4 concentration after TSH stimulation increased from 1.5 μg/dL (range, 0.2–2.7 μg/dL) to 2.7 μg/dL (range, 1.2–4.7 μg/dL); the recommended post-TSH T4 concentration was achieved by only 3 of 8 Sloughis. Hemoconcentration was found in 84.3% and hypoglobulinemia in 80.3%.

Conclusions and Clinical Importance: When evaluating Sloughis for hypothyroidism, veterinarians should be aware that these dogs have different thyroid hormone concentrations than nonsight hound dogs.

Key words: Free thyroxine; Globulins; Hemoconcentration; Sight hounds; Total thyroxine.

Hypothyroidism is a common endocrine disorder in dogs.[1] Diagnosis of hypothyroidism is established on the basis of clinical signs, physical examination, laboratory findings,[1,2] thyroid function testing,[3,4] and exclusion of nonthyroidal illness.[5] Ultrasonography may also be helpful in the diagnosis of hypothyroidism.[6–9] Baseline thyroid hormone concentrations can be influenced by many factors such as medication,[10–12] nonthyroidal disease,[13–15] racing and training,[16] and body size and age.[17] Serum thyroxine concentration also varies with breed. Greyhounds and Whippets have been reported to have lower total serum thyroxine concentrations than nonsight hound breeds.[18,19] In Greyhounds, a different response to TSH stimulation was also found compared with other breeds.[18]

Sloughis are the North African sight hounds, and they are still used for hunting in this region. In Europe, Sloughis are also used for racing. It is not known if Sloughis also have a lower normal range of serum thyroxine concentration. The purpose of this study was to determine if serum total thyroxine (tT4), free thyroxine (fT4), and canine thyroid stimulation hormone (cTSH) concentrations, and autoantibodies against thyroglobulin (TGAA) in Sloughis differ from those of other dog breeds and are similar to concentrations in Greyhounds and Whippets.

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Materials and Methods

Inclusion Criteria

Privately owned Sloughis from Germany, the Czech Republic, and France with no evidence of disease on history and physical examination were included in this study. On physical examination, attention was paid to general altitude, nutritional condition, heart and respiratory rate, rectal temperature, color and moisture of mucous membranes, capillary refill time, auscultation of the heart and respiratory tract as well as palpation of the abdomen, size and consistency of peripheral lymph nodes, hair, and skin. Dogs were fasted for a minimum of 12 hours before examination and blood sampling.

Exclusion Criteria

Females in heat, pregnancy, or lactation were not included in the study. Diestrus was not controlled and progesterone was not measured. CBC, serum biochemistry, and urinalysis were used to exclude nonthyroidal illness; any dog with abnormalities consistent with disease was excluded from the study. Medication with the exception of parasiticides was not permitted in the 3 months before sampling. Owners were asked specifically about administration of corticosteroids, estrus prevention drugs, thyroxine, and testosterone during the last 3 months.

Questionnaire

Relevant information was obtained using a standardized questionnaire. Owners were asked about signalment, origin, general health, estrus cycle of nonspayed females, medication administered in the past 3 months, exercise intolerance, heat seeking, and deworming schedules.

Thyroid Function Tests and Assays

Blood samples from all dogs were collected from cephalic or saphenous veins. After sampling, blood was permitted to clot at room temperature, and then centrifuged at 1,500 × *g* for 10 minutes. Serum was refrigerated to 4 °C and sent to the diagnostic laboratory[a] on the same or the following day. In all dogs, measurement of baseline tT4 by chemiluminescence immunoassay (CIA),[b] fT4 by CIA,[b] cTSH concentration by CIA,[b] and TGAA by CIA[c] was performed.

All assays were validated by the laboratory according to standard procedures, and the intra- and interassay coefficients of variation were 5.9 and 4.8% (tT4), 12.9 and 11.5 (fT4), 3.2 and 3.9% (cTSH), and 14.0 and 8.6% (TGAA), respectively. For the measurement of total thyroxine concentrations, a one-phase CIA[d] was used. The measurement of TSH concentration was also performed by a CIA.[d] Laboratory-specific reference ranges for T4 and TSH were 1.3–4.0 μg/dL and <0.5 ng/mL, respectively.[20,21] Serum for fT4 after equilibrium dialysis was frozen in the laboratory[a] to −70 °C within 48 hours after sampling until batch testing with the assay was performed. Determination of fT4 after ED was performed in a 2nd laboratory.[e] In[f] 8 Sloughis, a TSH stimulation test was performed by administering recombinant human (rh) TSH IV at a dose of 75 μg per dog before the start of racing season, according to a previously published protocol.[3] Serum for tT4 was collected before and 6 hours after IV administration of rhTSH and measured by tT4 CIA analysis. All owners granted permission to perform a TSH stimulation test in addition to the above-mentioned procedures, and this permission was obtained for 8 dogs. All but the values of fT4 after equilibrium dialysis were compared with the reference ranges established from the 95th percentile of 50 dogs (aged between 1 and 6 years) without evidence of clinical disease on history, physical examination, serum biochemistry, and CBC. Concentrations of fT4 after ED were compared with a reference population of 3,241 dogs with normal concentrations of total T3, total T4, and TSH and no detectable antibodies to T3 or T4. TSH stimulation test results were also interpreted according to more recent guidelines,[22] according to which an increase above 3.1 μg/dL or by at least 1.8 μg/dL is considered normal.

Other Tests

CBC and serum biochemistry were performed in every dog to minimize the risk missing nonthyroidal illness.

Statistical Evaluation

The various parameters in the Sloughis and control dogs were compared with an unpaired test. If the standard deviation was different between groups, a Welch's correction was used. For nonparametric data, a Mann-Whitney test was chosen. $P < .05$ was considered significant. To detect a correlation between the different thyroid results, a Pearson's test was performed for parametric data. A Spearman's test was chosen if the data were nonparametric. The reference range for tT4, fT4, and fT4 after ED and TSH in Sloughis was established from the 95th percentile of the dogs tested.

Results

Study Participants

Fifty-one healthy privately owned Sloughis (20 males, 29 females, 2 spayed females) from Germany (29 dogs), the Czech Republic (19), and France (3) were included in the study. Their age ranged from 1 to 12 years (mean and median, 4 years). Deworming practices varied widely from regular deworming every 3 months to dogs from large kennels (12–30 dogs/kennel) that were dewormed once every 2 years. Some dogs were apparently heat seeking in winter, but liked cool places in summer. None of the dogs showed exercise intolerance or lethargy.

CBC, Serum Biochemistry, and Urinalysis

On CBC and serum biochemistry, the dogs showed very pronounced hemoconcentration and high hemoglobin concentrations. Serum globulin concentrations were significantly decreased compared with the control group ($P < .0001$). Based on the normal reference range, hypoglobulinemia was present in 41 (80.4%) dogs. Hemoconcentration was found in 43 dogs (84.3%). PCV and serum globulin concentrations are presented in Table 1. Other biochemistry variables such as amylase, cholesterol, fructosamine, bilirubin, alkaline phosphatase, glutamate dehydrogenase, alanine transaminase, aspartate transaminase, albumin, total protein, and urea were in the normal range in all dogs. Six dogs had a very mildly increased serum creatinine concentrations, 4 mildly increased lipase activity (2 in combination with higher creatin kinase activity), and in 4 one of the serum electrolyte concentrations was mildly increased.

Thyroid Function Tests and Assays

The results of thyroid testing are presented in Table 2. The mean concentration of tT4 was significantly lower than that of control dogs ($P < .0001$). Thirty-two dogs (63%) had a tT4 lower than the reference range of 1.3–4.5 μg/dL. Similarly, the fT4 concentrations of Sloughis were significantly lower than those of normal dogs ($P < .0001$). However, only 8 dogs (16%) had fT4 lower than the reference range of 7.7–47.6 pmol/L. There was a significant correlation between tT4 and fT4 ($P < .0001$, $r = 0.9383$, Fig 1). Measurement of fT4 after ED was only possible in 41 dogs. When determined after ED, fT4 concentrations were significantly higher than those of the control group ($P < .0001$), but 3 dogs had concentrations below the normal reference range. There was a significant correlation between fT4 after ED and tT4 ($P < .0001$, $r = 0.807$) and between fT4 after ED and fT4 ($P < .0001$, $r = 0.852$). There was no correlation between tT4 and TSH ($P = .4038$, $r = 0.122$), between fT4 and TSH ($P = .4699$, $r = 0.1073$), and between fT4 after ED and TSH ($P = .083$, $r = -0.289$). There was a correlation between age and tT4 ($P = .0424$, $r = -0.311$) and age and fT4 ($P = .0442$, $r = -0.3199$, Fig 2). The cTSH concentrations of Sloughis were significantly higher than those of normal dogs ($P = .0138$), and the concentrations of 2 dogs were above the canine reference range. These 2 dogs had low tT4, but their fT4 and serum biochemistry

Table 1. PCV and serum globulin concentration of healthy Sloughis.

Parameter (Normal Range)	Mean Values ± SD	Median (Range)	Number of Dogs Outside the Normal Range
Globulins (25–45 g/L)	21.8 ± 3.5 g/L	21.1 (15.9–31.3) g/L	41
PCV (44–52%)	59 ± 5%	60 (48–68)%	43

Table 2. Descriptive statistics of thyroid values in 51 healthy Sloughis.

	tT4 (μg/dL) (Normal Range 1.3–4.5)	fT4 (pmol/L) (Normal Range 5.1–28.2)	ED fT4 (pmol/L) (Normal Range 6–42)	TSH (ng/mL) (Normal Range < 0.53)	T4 Pre-TSH (μg/dL) (Normal Range 1.3–4.5)	T4 Post-TSH (μg/dL) (Normal Range > 2.5)
Mean values ± SD	1.13 ± 0.65	11.00 ± 4.34	41.33 ± 26.88	0.22 ± 0.15	1.52 ± 0.95	2.74 ± 1.92
Median (range)	1.1 (0.1–2.7)	11.3 (2.4–19.1)	37 (3–101)	0.18 (0.03–0.71)	1.2 (0.2–2.7)	2.25 (1.2–4.7)
Confidence interval	0.95–1.13	9.73–12.22	33.77–48.9	0.18–0.26	0.73–2.32	1.64–3.84

tT4, total thyroxine concentrations; fT4, free thyroxine concentrations; ED fT4, free thyroxine concentrations after equilibrium dialysis; TSH, thyroid stimulation hormone; SD, standard deviation.

(with exception of hypoglobulinemia) was normal. A TSH stimulation test was not performed in these 2 dogs. Pre- and post-TSH stimulation test thyroxine concentrations were below the reference range of the laboratory for normal pet dogs in 5 of the 8 dogs (Fig 3). All 45 dogs had low tT4, but in 3/4 dogs the fT4 was normal and in the 2 dogs in which fT4 after ED was determined, it was normal as well. None of the dogs had any clinical abnormalities or had a history of health problems or abnormal behavior. Besides a mild increase of lipase activity in one and of serum creatinine concentration in another, all of these dogs had normal serum biochemistry and their red blood cell counts were normal as well. None of the dogs had detectable antibodies against thyroglobulin.

Discussion

In our study, Sloughis had significantly lower serum concentrations of tT4 when compared with mean values and standard deviations of normal dogs supplied by the laboratory.[a] Free thyroxine concentrations were also significantly lower than those of other dogs when measured by CIA. In contrast, when measured after ED, fT4 concentrations were in the normal range or even increased compared with healthy controls. Historically, none of the owners reported clinical signs of hypothyroidism, and clinical examination did not identify any changes suggestive for this disorder. Thus, in Sloughis we could confirm lower tT4 and fT4 concentrations compared with other nonsight hound dogs, similar to what has been reported in Greyhounds[18] and Whippets.[19]

We chose Sloughis from 3 different countries to ensure wide genetic diversity. The dogs in general were of quiet disposition, but no evidence of hypothyroidism such as exercise intolerance, recurrent infections, or weight gain was present. Some dogs were apparently heat seeking in winter. Those dogs, however, sought cool places in the summer; thus this was considered normal behavior rather than a sign of possible hypothyroidism. Most of the thyroxine in the bloodstream is bound to carrier proteins. The most important such protein is thyroid hormone-binding globulin, but albumin and some lipoproteins may also bind thyroxine. If the higher concentration of fT4 after ED is caused by the low total globulin concentration seen in this breed with a possible associated low concentration of thyroid hormone-binding globulin or if it is a result of other causes is unknown. Other possible explanations for the lower thyroid hormone concentrations include a higher efficiency in converting T4 to T3 or a more sensitive feedback mechanism resulting in decreased concentrations as suggested for Greyhounds.[18]

In 8 Sloughis in which TSH stimulation tests with rhTSH were performed, normal stimulation was seen in 4 dogs. Four dogs showed post-TSH thyroxine concentrations below the reference range. According to more recent recommendations, 5/8 dogs showed insufficient stimulation.[22] The recommendation is a post-TSH T4 concentration of 3.1 μg/dL or at least an increase of 1.8 μg/dL. The lower thyroxine concentrations post-TSH may be a result of the same reason as the low basal thyroxine concentrations. Again, lower plasma globulin concentration, higher efficiency in conversion of T4 to T3

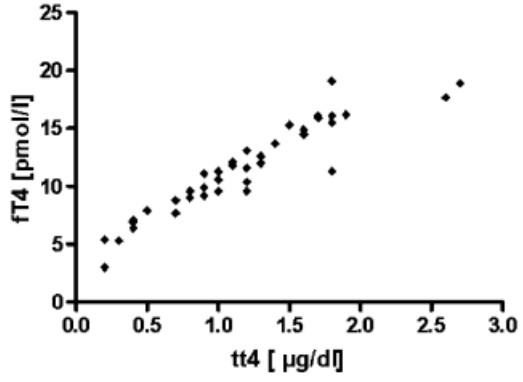

Fig 1. Correlation between total thyroxine (tT4) and free thyroxine (fT4) in the Sloughis included in the study.

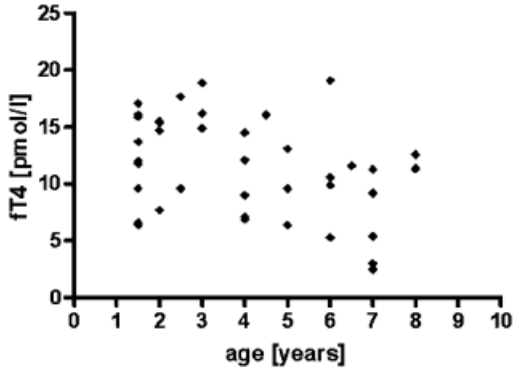

Fig 2. Correlation between free thyroxine (fT4) and age of Sloughis included in the study.

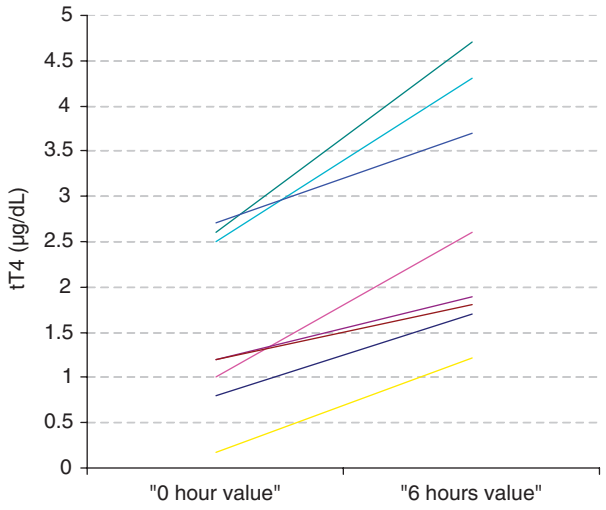

Fig 3. Total thyroxine concentrations before and 6 hours after IV administration of rhTSH at a dose of 75 µg per dog in 8 healthy Sloughis.

or more sensitive feedback mechanisms in Sloughis compared with other breeds may be responsible for these results. Alternatively, one or all of these dogs may have been hypothyroid. However, their history, clinical examination, biochemistry, and CBC did not provide findings any evidence of hypothyroidism. Antibodies against thyroglobulin were also not detected in any of the dogs. In previous studies, such antibodies were detected in 36 and 53% of hypothyroid dogs and were considered more prominent in the early stages of the disease.[23,24] Thus, one would expect these antithyroglobulin antibodies in at least some of the Sloughis if preclinical hypothyroidism was indeed the cause of the low thyroxine concentrations. Furthermore, none of these Sloughis showed decreased fT4 after ED. In light of the lower tT4 concentrations of the other Sloughis evaluated in this study and no other evidence of the disease, we believe it unlikely that these dogs are hypothyroid. In the study by Gaughan and Bruyette, thyroxine concentrations pre- and post-bovine TSH were lower in Greyhounds than in non-Greyhound breeds and very similar to the results found in the Sloughis of this study. The number of dogs tested was small, and rhTSH was used as compared with bovine TSH in the Greyhounds of the previous study. However, in light of the low tT4 concentrations, it seems likely that these findings would be similar in a larger group of dogs. Sloughis and Greyhounds (both sight hounds) may have similar thyroxine metabolism.

In our study, CBC and biochemistry results from healthy Sloughis resembled those reported for Greyhounds (ie, hemoconcentration and low globulin concentrations).[18,25] Hemoconcentration may be seen with dehydration and in that case typically is associated with hyperproteinemia and hyperalbuminemia. Changes in serum biochemistry such as azotemia may also occur with dehydration. Because neither clinical examination nor changes on serum biochemistry provided evidence for dehydration, it seems unlikely that dehydration was the cause of hemoconcentration in the Sloughis in this study. The underlying mechanism for the difference in serum protein concentration has not been determined. Ilkiw[26] suggested that the low protein concentration in Greyhounds could be the result of chronic plasma volume expansion associated with chronic conditioning and training. Greyhounds have significantly higher PCV and blood viscosity than do other dog breeds. Hypoproteinemia may be an adaptive mechanism to decrease serum viscosity. Genetic factors seem more likely as possible causes for these changes.

Ultrasonographic examination of thyroid glands in Sloughis may be an additional tool to differentiate hypothyroid from normal dogs. Ultrasonographic examination evaluating the size and the echogenity of the thyroid gland should identify hypoechoic areas with lymphocytic thyroiditis, as described.[6,7]

In summary, significantly decreased tT4, fT4 concentrations and increased TSH concentrations were seen in Sloughis compared with nonsight hound breeds. Hematological changes such as high PCV and low serum globulin concentration also were detected. Veterinarians should be aware of these differences when evaluating serum biochemistry, CBC, and thyroid function test results in Sloughis.

Footnotes

[a] Laboklin GmbH & Co KG, Bad Kissingen, Germany
[b] Immulite 2000, Siemens, Munich, Germany
[c] Advia Centaur, Siemens
[d] Immulite Canine Total T4 Assay, Diagnostic Products Corporation, Los Angeles, CA
[e] Michigan State University, East Lansing, MI
[f] Tierklinik Birkenfeld, Birkenfeld, Germany

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References

1. Scott DW, Miller WH, Griffin CE. Small Animal Dermatology, 6th ed. Philadelphia, PA: WB Saunders; 2001:851–865.
2. Ferguson DC. Update on diagnosis of canine hypothyroidism. Vet Clin North Am Small Anim Pract 1994;24:515–539.
3. Boretti FS, Sieber-Ruckstuhl NS, Favrot C, et al. Evaluation of recombinant human thyroid-stimulating hormone to test thyroid function in dogs suspected of having hypothyroidism. Am J Vet Res 2006;67:2012–2016.
4. Frank LA. Comparison of thyrotropin-releasing hormone (TRH) to thyrotropin (TSH) stimulation for evaluating thyroid function in dogs. J Am Anim Hosp Assoc 1996;32:481–487.
5. Kantrowitz LB, Peterson ME, Trepanier LA, et al. Serum total thyroxine, total triiodothyronine, free thyroxine, and thyrotropin

concentrations in epileptic dogs treated with anticonvulsants. J Am Vet Med Assoc 1999;214:1804–1808.

6. Bromel C, Pollard RE, Kass PH, et al. Ultrasonographic evaluation of the thyroid gland in healthy, hypothyroid, and euthyroid Golden Retrievers with nonthyroidal illness. J Vet Intern Med 2005; 19:499–506.

7. Reese S, Breyer U, Deeg C, et al. Thyroid sonography as an effective tool to discriminate between euthyroid sick and hypothyroid dogs. J Vet Intern Med 2005;19:491–498.

8. Taeymans O, Daminet S, Duchateau L, et al. Pre- and post-treatment ultrasonography in hypothyroid dogs. Vet Radiol Ultrasound 2007;48:262–269.

9. Taeymans O, Duchateau L, Schreurs E, et al. Intra- and interobserver variability of ultrasonographic measurements of the thyroid gland in healthy Beagles. Vet Radiol Ultrasound 2005; 46:139–142.

10. Daminet S, Ferguson DC. Influence of drugs on thyroid function in dogs. J Vet Intern Med 2003;17:463–472.

11. Frank LA, Hnilica KA, May ER, et al. Effects of sulfamethoxazole-trimethoprim on thyroid function in dogs. Am J Vet Res 2005;66:256–259.

12. Gulikers KP, Panciera DL. Evaluation of the effects of clomipramine on canine thyroid function tests. J Vet Intern Med 2003;17:44–49.

13. Kraft W, Dietl A. Total thyroxine (T4) and free thyroxine (FT4) in hypothyroidism and nonthyroid diseases of dogs. Tierarztl Prax 1994;22:472–479.

14. Nelson RW, Ihle SL, Feldman EC, et al. Serum free thyroxine concentration in healthy dogs, dogs with hypothyroidism, and euthyroid dogs with concurrent illness. J Am Vet Med Assoc 1991; 198:1401–1407.

15. von Klopmann T, Boettcher IC, Rotermund A, et al. Euthyroid sick syndrome in dogs with idiopathic epilepsy before treatment with anticonvulsant drugs. J Vet Intern Med 2006; 20:516–522.

16. Hill RC, Fox LE, Lewis DD, et al. Effects of racing and training on serum thyroid hormone concentrations in racing Greyhounds. Am J Vet Res 2001;62:1969–1972.

17. Reimers TJ, Lawler DF, Sutaria PM, et al. Effects of age, sex, and body size on serum concentrations of thyroid and adrenocortical hormones in dogs. Am J Vet Res 1990;51:454–457.

18. Gaughan KR, Bruyette DS. Thyroid function testing in Greyhounds. Am J Vet Res 2001;62:1130–1133.

19. Van Geffen C. Serum thyroid hormone concentrations and thyroglobulin autoantibodies in trained and non-trained healthy whippets. Vet J 2006;172:135–140.

20. Hoppen HO, Lohmann P, Schlote S, et al. Die Messung von caninem TSH zur Diagnostik der Hypothyreose des Hundes. Der praktische Tierarzt 1997;78:13–17.

21. Koch A. Untersuchungen der Prolaktinsekretion im Zusammenhang mit der Freisetzung von LH und Testosteron sowie Thyroxin und Thyrotropin bei Beagle Rüden. Thesis. Institut für Reproduktionsmedizin. Hannover, Germany: Tierärztliche Hochschule 2004.

22. Daminet S, Fifle L, Paradis M, et al. Use of recombinant human thyroid-stimulating hormone for thyrotropin stimulation test in healthy, hypothyroid and euthyroid sick dogs. Can Vet J 2007;48:1273–1279.

23. Dixon RM, Mooney CT. Canine serum thyroglobulin autoantibodies in health, hypothyroidism and non-thyroidal illness. Res Vet Sci 1999;66:243–246.

24. Lee JY, Uzuka Y, Tanabe S, et al. Prevalence of thyroglobulin autoantibodies detected by enzyme-linked immunosorbent assay of canine serum in hypothyroid, obese and healthy dogs in Japan. Res Vet Sci 2004;76:129–132.

25. Sullivan PS, Evans HL, McDonald TP. Platelet concentration and hemoglobin function in Greyhounds. J Am Med Vet Assoc 1994;205:838–841.

26. Ilkiw JE, Davis PE, Church DB. Hematologic, biochemical, blood-gas, and acid-base values in Greyhounds before and after exercise. Am J Vet Res 1989;50:583–586.