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Factors influencing serum concentrations of total thyroxine, free thyroxine and thyrotropin in healthy dogs of various breeds*)

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Summary

The aim of this article is to obtain the correct specification of reference ranges of TT4, fT4 and cTSH with regard to the influence of physiological factors on thyroid gland functioning, including breed size, age and season.

The reference range for TT4 was divided according to the size of the dog breeds. In large breeds, over 27 kg of live weight, it should be represented by the interval TT4 = 17-43 nmol/l; in medium size breeds, from 12 to 27 kg of live weight, it should be TT4 = 23-50 nmol/l and in small breeds, up to 12 kg of live weight, the reference range should move within TT4 = 27-55 nmol/l. The reference range of free thyroxin does not depend on the size of the breed and it is represented by a uniform standard: fT4 = 10-22 pmol/l. The reference level for cTSH in healthy dogs should not exceed cTSH = 0-0.35 ng/ml.

Keywords: dog, hypothyroidism, euthyroid syndrome

The works of various authors document differing influences on serum thyroidal hormone concentration levels in dogs, including breed size and age, but also drug application or nonthyroidal diseases (2, 7, 17). Their aim has been to use this knowledge in hypothyroidism diagnostics and its differentiation from euthyroid sick syndrome. In this diagnostics for dogs, many authors most frequently use serum total thyroxine (TT4), free thyroxine (fT4) and canine thyroid stimulating hormone (cTSH) concentration monitoring as a routine necessity (1, 4). The evaluation of these marker concentrations requires their exact reference ranges that would simultaneously respect extrathyroidal factors which are physiological in dogs and also influence TT4, fT4 and cTSH concentrations in healthy dogs.

Dogs size, season and animal age are the most frequent factors influencing thyroid hormones and cTSH concentrations in healthy dogs (5, 10). The evaluation of results of endocrinological examinations should be done according reference ranges which include these physiological extrathyroidal factors. The aim of this study was to suggest equivalent TT4, FT4 and cTSH reference ranges for dogs in relation to physiological factors like breed size, season and age which influence their concentrations also in healthy individuals.

Material and methods

Our study includes the monitoring results of 292 healthy dogs based on detailed clinical examinations and repeated hematological, biochemical and endocrinological marker evaluations. Obese dogs and females with serum progesterone concentrations exceeding 0.5 ng/ml were excluded. Dogs were divided into 3 groups based on body weight.

The most frequently monitored healthy dogs (n = 128) belonged to the big breeds with body weight higher than 27 kg. The most dominant were dogs of the following breeds: German Shepherd (n = 49), Golden Retriever (n = 9), Labrador Retriever (n = 9), Boxer (n = 8) and Rottweiler (n = 9). Less frequent were dogs of these breeds: Doberman Pincher (n = 7), mixed (n = 7), Rhodesian Ridgeback (n = 5), Giant Shnauzer (n = 3), Siberian Husky (n = 4),

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Briard (n = 4), Bobtail (n = 5), Great Dane (n = 3), Newfounland (n = 4) and Bernese Mountain Dog (n = 2). The middle-size breed group was represented by dogs with a body weight ranging from 12 to 27 kg with a total count of 77 dogs. This group included dogs of the following breeds: English Cocker Spaniel (n = 28), American Cocker (n = 7), Mixed (n = 7), English Springer Spaniel (n = 5), English Bulldog (n = 4), Beagle (n = 2), Bull-Terrier (n = 3), Dalmatian (n = 5), Staffordshire Terrier (n = 5), Kerry blue Terrier (n = 3), Keeshound (n = 5) and Shar-pei (n = 3). Dogs with low body weight, up to 12 kg (n = 87), represented the small breeds group and the most frequent breeds were: Poodle (n = 19), Dachshund (n = 17), Maltese dog (n = 9), Toy Keeshound (n = 8) and Yorkshire Terrier (n = 6). Less frequent were dogs of the breeds: Pug (n = 5), Mixed (n = 5), Pekingese (n = 4), Miniature Pincher (n = 4), Cavalier King Charles Spaniel (n = 2), Chihuahua (n = 3), Lhasa-apso (n = 2) and West-Highland White Terrier (n = 3).

The age of dogs of all breeds ranged from 6 months to 10 years. Individuals for monitoring were strictly selected after repeatedly reevaluated examinations of their clinical status, haematological and biochemical blood examinations during several years. The dogs were also considered as healthy on the basis of others diagnostic procedures (radiography, ultrasonography). There were 150 females and 142 males among them.

Serum TT4 and fT4 concentrations were measured with a radioimmunoassay and serum cTSH concentration with an immunoradiometric assay validated for use on serum from dogs (Coat-A-Count Canine TSH, IRMA, Diagnostic Products, Los Angeles, CA).

The intra-assay precision for TT4 was specified by a 10 time analysis of samples. The variation coefficients for TT4 were found below or equal to 6.2%. The interassay variation coefficients for TT4 were found below or equal to 8.6%. Sampels were assayed in duplicate in 10 different series.

The intra-assay variation coefficients were found below or equal to 8.3% for fT4 serum samples, which were assayed 20 times in the same series. The inter-assay variation coefficients were found below or equal to 7.5% for fT4 serum samples, which were assayed in duplicate in 15 different series.

The intra-assay variation coefficients were 9.5%, 3.6%, 4.0% and 2.0% for cTSH values of 0.21 ng/ml, 0.28 ng/ml, 0.50 ng/ml and 3.53 ng/ml respectively. The interassay variation coefficients were 9.5%, 8.8%, 5.1% and 3.9% for cTSH values of 0.21 ng/ml, 0.34 ng/ml, 1.36 ng/ml and 2.59 ng/ml, respectively.

Two-way ANOVA was used for statistical comparison of basal serum TT4 or fT4 and cTSH concentration between breeds depending on the season. The value of $p < 0.05$ was considered significant. One-way ANOVA was used for comparison of TT4, FT4 and cTSH concentrations depending on the age of various dog breeds.

Seasons were divided by months, where March, April and May were considered as spring and June, July and August as summer. Fall was represented by September, October and November and winter by December, January and February.

Blood samples were in most of the cases taken between 10.00 a.m. to 03.00 p.m. after 12 hours of fasting by venipuncture of v. cephalica antebrachii.

Results and discussion

Serum TT4 concentrations. In the group of healthy dogs of big, medium and small breeds, serum TT4 concentrations ranged from 20.4 to 43.9 nmol/l, 26.8-51.1 nmol/l and 29.9-50.8 nmol/l, respectively. The average concentrations of TT4 in the group of big, medium and small breed dogs were 30.19 nmol/l (standard deviation sd = 6.64), 36.8 nmol/l (sd = 7.3), and 40.18 nmol/l (sd = 6.89) respectively.

The comparison of listed average TT4 concentrations in various weight groups of dogs clearly approved its increasing tendency in relation to decreasing body weight of the breed. The statistic analysis of TT4 concentrations of healthy dogs revealed a statistically significant difference between weight groups of dogs on the level $\alpha = 0.05$.

Data evaluation of TT4 examinations by seasons confirmed changes in their concentrations during the year. The lowest serum TT4 concentration occurred in the group of healthy dogs of the big breeds in the summer (TT4 = 25.52 nmol/l) and the highest one in spring (tT4 = 30.32 nmol/l) and in winter (TT4 = 29.82 nmol/l). The lowest serum TT4 concentrations occurred in the group of healthy dogs of medium and small breeds in the summer (TT4 = 32.72 nmol/l), respectively (TT4 = 36.89 nmol/l), similarly as in big breed dogs. The highest serum TT4 concentrations occurred in the group of healthy medium and small breeds in winter (TT4 = 36.66 nmol/l), respectively (TT4 = 39.82 nmol/l). Generally, serum TT4 concentrations of clinicaly healthy dogs in various weight groups were lower in the summer in comparison to concentrations in the fall and winter (fig. 1).

The statistical analysis of serum TT4 concentrations of big, medium and small breed dogs during the year by seasons revealed a significant difference on the level $\alpha = 0.05$ also depending on the season.

The evaluation of serum TT4 concentrations depending on age revealed the highest average level in young

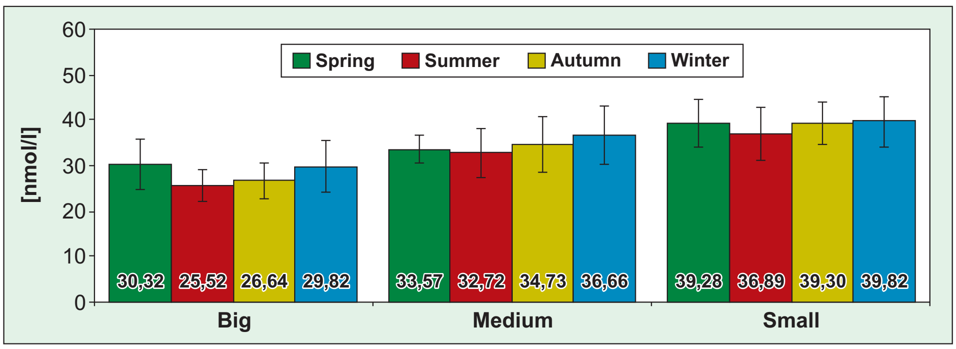

Fig. 1. Average serum TT4 concentrations in healthy dogs depending on the season in big, medium and small breeds with labelled standard deviations

dogs up to 1-year-of-age and in 1-year-old ones in the group of big (TT4 = 35.55 nmol/l), medium (TT4 = 43.43 nmol/l) and small breed dogs (TT4 = 47.86 nmol/l) respectively. The lowest average concentrations of TT4 were in 10-year-old dogs of big (TT4 = 25.57 nmol/l), medium (TT4 = 30.17 nmol/l) and small breeds (TT4 = 33.34 nmol/l) respectively. The comparison of average serum TT4 concentrations in healthy dogs of various breeds in relation to age is shown in fig. 2 with clearly demonstrated different physiological TT4 levels in big, medium and small dog breeds.

Average serum TT4 concentrations in big, medium and small breeds of dogs together with standard deviations were used for the calculation of 2 differences from the average ($2s^-$ a $2s^+$), in which 95.54% of the results of all TT4 data measurements in healthy dogs is found for each weight group.

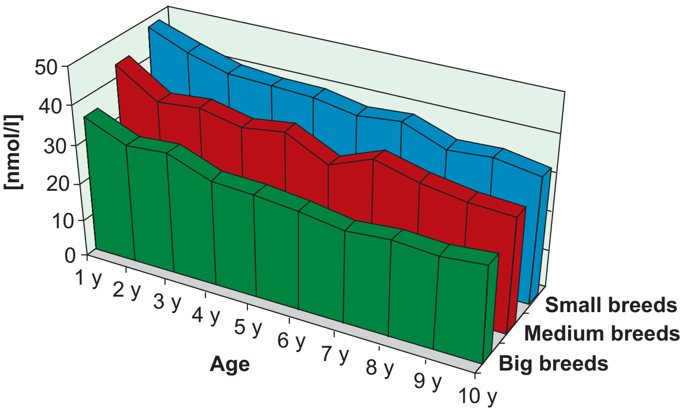

Fig. 2. Comparison of average serum TT4 concentrations in healthy dogs of various breeds depending on age

Therefore, it is possible to predict that the range of 2 standard deviations ($2s^-$ a $2s^+$) from the average in big (TT4 = 30.18 nmol/l), medium (TT4 = 36.8 nmol/l) and small breed dogs (TT4 = 40.18 nmol/l), i.e. the intervals 16.92-43.47 nmol/l, 22.19-51.42 nmol/l and 26.40-53.96 nmol/l respectively, will represent the most probable physiological TT4 range in healthy medium and small breeds of dogs.

The presented intervals of 2 deviations from TT4 average in various weight groups of big, medium and small breeds includes also average TT4 concentrations obtained by their concentration measurements depending on age. On the base of presented TT4 results, we can suggest reference ranges differentiated on the size of dog breeds as follows: reference TT4 range for big-breed dogs: TT4 = 17-43 nmol/l; reference TT4 range for medium-breed dogs: TT4 = 23-50 nmol/l; reference TT4 range for small-breed dogs: TT4 = 27-55 nmol/l.

Serum fT4 concentrations. Serum fT4 concentrations were monitored in groups of big (n = 83), medium (n = 57) and small (n = 71) breed dogs and their concentration values ranged from 10.1 to 22.6 pmol/l, 8.9-19.8 pmol/l and 10.2-25.3 pmol/l respectively. The average concentrations of fT4 were 14.09 pmol/l (sd = 2.11), 14.92 pmol/l (sd = 2.74) and 15.75 pmol/l (sd = 2.74).

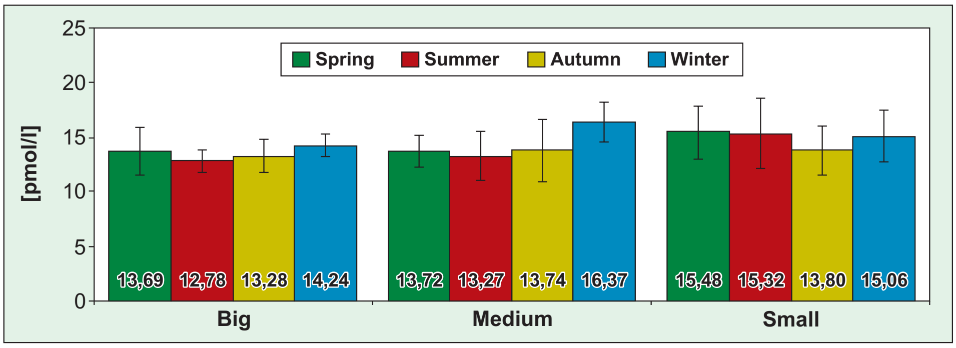

Fig. 3. Average serum fT4 concentrations in healthy dogs in relation to the season in big, medium and small breeds with labelled standard deviations

The comparison of listed average fT4 concentrations in various weight groups confirmed its increased tendency in relation to decreasing breed weight, but a statistical evaluation did not show significant differences.

The evaluation of the results of fT4 measurements by seasons confirmed that serum fT4 concentration fluctuated during the year from the lowest values in the summer to the highest values in the winter, but only in big- and medium-breed dogs. In small-breed dogs, this dynamic in values fluctuation was not confirmed (fig. 3). A statistically significant difference in average fT4 concentrations was also noticed during the year by seasons.

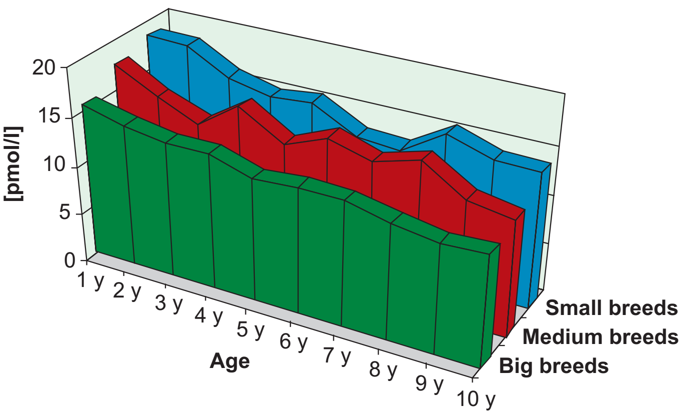

Fig. 4. Comparison of average serum fT4 concentrations of healthy dogs of various breeds in relation to age

The evaluation of serum fT4 concentrations depending on age revealed the highest average level in young dogs up to 1-year-of-age and in 1-year-old dogs in the group of big (fT4 = 15.75 pmol/l), medium (fT4 = 17.69 pmol/l) and small breed dogs (fT4 = 18.62 pmol/l) respectively. The lowest average concentrations of fT4 were in 10-year-old dogs of big (fT4 = 11.50 pmol/l), medium (fT4 = 12.05 pmol/l) and small breeds (fT4 = 14.15 pmol/l) respectively. See fig. 4 for the comparison of average serum fT4 concentrations in various weight groups of dogs depending on age.

The average serum fT4 concentrations in big, medium and small breeds together with standard deviations were used for calculating the probable physiological fT4 range in healthy dogs. According to the probability division of normal splitting up, 95.45% of the results from all fT4 measured values in healthy dogs of big, medium and small breeds are found in the range of 2 deviations ($2s^-$ and $2s^+$) around average value.

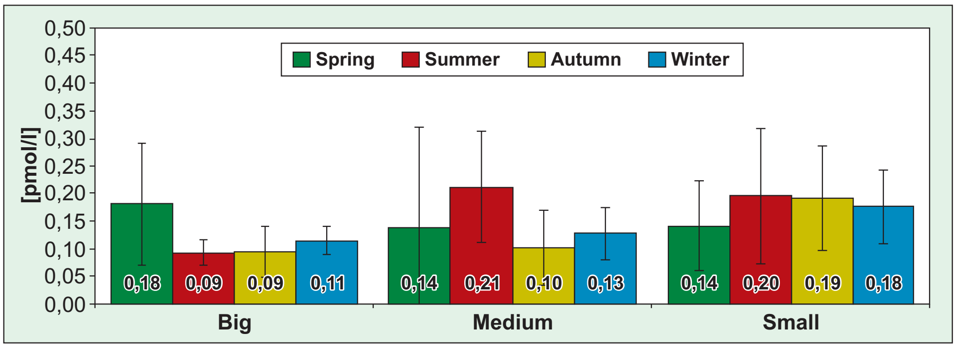

Fig. 5. Average serum cTSH concentrations in healthy dogs depending on the season in big, medium and small breeds with labelled standard deviations

Therefore, it is possible to predict that the range of 2 standard deviations ($2s^-$ a $2s^+$) from the average in big (fT4 = 14.09 pmol/l), medium (fT4 = 14.92 pmol/l) and small breeds dogs (fT4 = 15.75 pmol/l), i.e. the intervals 9.86-18.32 pmol/l, 9.44-20.4 pmol/l and 10.28-21.23 pmol/l respectively, will represent the most probable physiological fT4 range in healthy dogs of the specified weight.

As the statistical comparison of results of fT4 concentrations did not confirm any significant differences between breeds, it was not neccesary to determine the physiological range for each weight group of dogs in particular, but it could be defined jointly for all breeds. The proposed probable range for serum fT4 concentrations for all breeds could be the interval of: fT4 = 10-22 pmol/l.

Serum cTSH concentrations. Serum cTSH concentrations were evaluated in the groups of big (n = 46), medium (n = 35) and small (n = 54) breeds of dogs and the average concentrations were 0.121 ng/ml (sd = 0.058), 0.14 ng/ml (sd = 0.08) and 0.16 ng/ml (sd = 0.09) respectively. The comparison of averge serum cTSH concentrations showed a moderate increasing tendency of cTSH values in relation to decreasing body weight of the breed, but the monitoring of average cTSH concentrations during the year by seasons in all dog breeds brought very mixed results confirming that the seasons do not influence cTSH concentrations in healthy dogs (fig. 5). The statistical analysis did not show any significant difference either when comparing weight groups, or seasons.

When assessing serum cTSH concentrations in all dogs breeds in relation to age, we found the lowest average concentration in young dogs up to 1-year-of-age and in 1-year-old dogs and the same held true for big (cTSH = 0.11 ng/ml,), medium (cTSH = 0.12 ng/ml) and small breeds (cTSH = 0.11 ng/ml). The highest average cTSH concentrations were demonstrated in the oldest dogs aged 10 years and in the same manner in all dog breeds – in big (cTSH = 0.29 ng/ml), medium (cTSH = 0.24 ng/ml) and in small breeds (cTSH = 0.20 ng/ml), too (fig. 6).

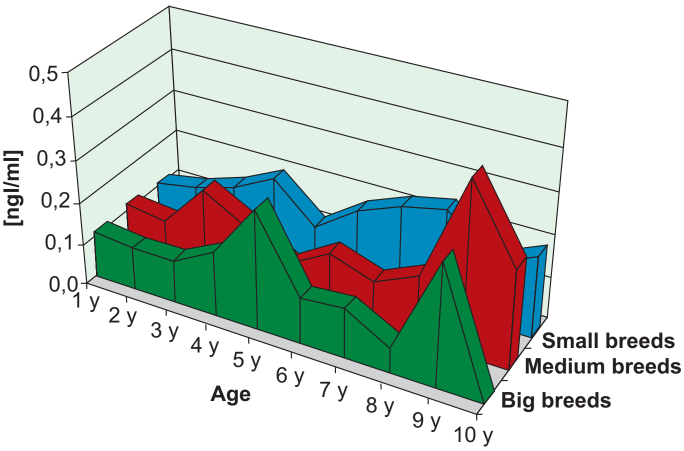

Fig. 6. Comparison of average serum cTSH concentrations of healthy dogs of various breeds in relation to age

Assuming that according to probability the division of normal splitting up, 95.45% results of all measured values in big (cTSH = 0.121 ng/ml), medium (cTSH = 0.14 ng/ml) and small (cTSH = 0.16 ng/ml) breeds are found within the range of 2 deviations ($2s^-$ a $2s^+$), then intervals (0.0-0.24 ng/ml; 0.0-0.31 ng/ml; 0.0-0.34 ng/ml) represent the most probable range of physiological cTSH values in healthy dogs of big, medium and small breeds.

Based on the above mentioned results, we can propose reference serum cTSH concentration ranges in healthy dogs of all breeds as follows: cTSH = 0.0--0.35 ng/ml.

The reported monitoring of serum TT4, fT4 and cTSH concentrations in healthy dogs brought appropriate information about their physiological levels and also considered factors like breed size, age and season. The statistical analysis of serum TT4 concentration results showed the highest differences in their levels in relation to breed size and season.

Many authors try to overbridge the large differences in the physiological TT4 levels among breeds with large-scale reference range in healthy dogs. Ramsey (21) considers as physiological TT4 results of examinations in the range TT4 = 25-80 nmol/l; Daminet and al. (2) uses TT4 range TT4 = 15-67 nmol/l. In her study Kolevská et al. (13) used reference TT4 levels TT4 = 18-39 nmol/l in healthy dogs. The application of such a large-scale (wide standard) TT4 range has not proved good in our practice, because the results on lower range (in the lower limit of the standard), e.g. TT4 = 18.5 nmol/l, are suitable for dogs of big breeds, but according our observations, they might be insufficient for healthy dogs of small breeds. The results of TT4 concentrations above 40 nmol/l, on the contrary, were common findings in dogs of small breeds, but they occurred very rarely in dogs of large breeds, and then only in puppies. Several authors support the necessity of dividing referene range by size of the breed (6, 14, 18).

Many authors describe the effect of thyroid hormones that influence body growth, tissue differentiation and several metabolic processes. Thyroid gland hormones also increase oxygen utilization in tissues (except CNS, adenohypophysis, testicules, uterus and lymphatic tissue), the production of 2.3–difosfoglycerate in erythrocytes, that enhances oxygen release from haemoglobin, and they also stimulate the generation of warmth in the organism (8). Therefore, the assumption that TT4 levels should vary depending on ambient temperature is understandable and, moreover, they should also mirror the season (5). Other authors also monitored serum TT4 and fT4 concentrations in dogs of various breeds during the year and reported on their fluctuation within each month. Even though TT4 concentrations during the year were not in direct correlation with ambient temperatures in each month, the highest TT4 concentrations appeared in November and January (10). Our monitoring also focused on this fact, where serum TT4 concentration results during the year obviously showed the lowest TT4 levels in the summer and, conversely, the highest TT4 levels in winter in all weight groups of dogs.

A statistical analysis of TT4 concentrations of healthy dogs obviously showed marked and significant differences among the various weight groups of dogs, and simultaneously showed statistically significant differences while comparing TT4 levels during the year by seasons, too. It also confirmed the necessity of the division of reference ranges in the evaluation of TT4 results according the breed size.

In puppies of small breeds, the upper reference range may be moved up to 60 nmol/l. However, excessively increased serum TT4 concentrations above the mentioned range should confirm TT4 (T4AA) the presence of antibodies (19, 23).

The monitoring of serum fT4 concentrations in various weight groups of dogs did not bring any significant differences as to levels among breeds or seasons of the year. Hence the reference range was not divided, but given jointly for all breeds. The presented results clearly showed that serum fT4 concentrations in dogs were not influenced by so many factors as TT4 concentrations. Therefore serum fT4 measurement is more recommended for diagnostics of hypothyroidism than for TT4 measurement which is used only as a screening method. The possible combination of serum TT4, fT4 and cTSH concentration evaluation is the best approach to thyroid function monitoring and thereby also the proper differential diagnostics between true hypothyroidism and euthyroid sick syndrome (4, 11, 16).

The monitoring of serum cTSH concentrations in our dogs confirmed the fact that their levels are not influenced either by breed size, or by season. Their concentrations can be increased by age in relation to the physiological decreasing of thyroid hormon concentrations in the blood.

The results of evaluations obtained by other authors confirm very low serum cTSH concentrations in healthy dogs, which is compatible with our results (20, 22). For the evaluation of serum cTSH concentrations, authors in different studies specified various reference ranges, limiting cTSH concentrations in healthy dogs. Dixon and Money (4) report about a range of cTSH = up to 0.68 ng/ml which should not be exceeded by healthy or euthyroid dogs. On the other hand, other studies found a very low average of cTSH concentrations in hypothyroid dogs (cTSH = 0.47 ng/ml) and the reference range established for healthy dogs was only cTSH = 0.02-0.45 ng/ml (22). Nachreiner et al. (15) lowered the physiological cTSH range for cTSH = to 0.41 ng/ml and Boretti and Reusch (1) give a reference cTSH range up to 0.40 ng/ml, when sensitivity of the test was raised to 73% and specificity to 100%. The presented reference values of various studies condition various sensitivity and specificity of cTSH tests in the named studies, where the authors admit the existence of false positive and negative results. This means that in a small percentage of hypothyroid dogs it is possible to detect low cTSH concentrations or increased cTSH values in healthy and/or euthyroid dogs. Several reasons have been suggested to explain the inappropriately low cTSH concentrations in hypothyroid dogs. Circadian and pulsatile variations in hypothalamic TSH was confirmed in a model of experimentally induced primary canine hypothyroidism (12). Thus it seems unlikely to be the primary reason for the limited sensitivity of cTSH determination. Secondary hypothyroidism may be diagnosed in a few hypothyroid dogs with low cTSH. However, pituitary failure is thought to occur only in about 5% of hypothyroid dogs (6, 9).

The reason for high cTSH concentrations in healthy or in euthyroid dogs without marked clinical signs of hypothyroidism may be the so called subclinical form of hypothyroidism, when serum TT4 and fT4 concentrations in physiological ranges are still maintained, but to the detriment of stimulation action of adenohypophysal cTSH (1, 22). On the basis of above mentioned, we can also agree with other authors that serum cTSH concentrations are influenced by several of the mentioned factors and, therefore, the determination of physiological cTSH ranges is an important part of correct hypothyroidism diagnostics. If the fact is considered that in subclinical forms we can detect increased serum cTSH concentrations with normal TT4 levels, then the physiological cTSH range in healthy dogs can be defined as cTSH = 0.0-0.4 ng/ml, as other authors also report (1). Reported monitored results, however, indicated the fact that in healthy dogs cTHS values should not exceed the value of cTSH = 0.35 ng/ml. Unexpectedly high serum cTSH values in healthy dogs might have resulted from the recovery from illness or concurrent potentiated sulphonamide therapy. CTSH serum concentrations exceeding the given range of cTSH = 0.4 ng/ml may – most probably – show hypothyreosis in its early stages and should be repeatedly tested.

The presented monitoring of serum thyroid hormone levels and cTSH in healthy dogs show their predicted optimal physiological levels that take into account breed size, age and season influence. The results indicate the fact that serum TT4 concentrations in dogs should be evaluated individually, not only with reference to breed size, but to the season as well, and this would allow a correct determination of the dynamics of thyroid gland functioning in the dog.

References

1. *Boretti F. S., Reusch C. E.*: Endogenous TSH in the diagnosis of hypothyroidism in dogs. Schweiz. Arch. Tierheilk. 2004, 146, 183-188.
2. *Daminet S., Ferguson D. C.*: Influence of Drug on Thyroid Function in Dogs. J. Vet. Intern. Med. 2003, 17, 463-472.
3. *Daminet S., Jeusette I., Duchateau L., Diez M., Van Den Maele I., De Rick A.*: Evaluation of Thyroid Function in Obese Dogs and Dogs Undergoing a Weight Loss Protocol. J. Vet. Med. 2003, 50, 213-218.
4. *Dixon R. M., Mooney C. T.*: Evaluation of serum free thyroxin and thyrotropin concentration in the diagnosis of canine hypothyroidism. J. Small Anim. Pract. 1999, 40, 72-78.
5. *Eiji O., Katsayoshi Y., Yoji U., Shigeyuki T., Tkao S., Takuo I.*: Seasonal changes in serum total thyroxine, free thyroxine and canine thyroid response hormone in clinically healthy beagles in Hokkaido. J. Vet. Med. Sci. 2001, 63, 1241-1243.
6. *Feldman E. C., Nelson R. W.*: Canine and Feline Endocrinology and Reproduction. Saunders W. B., St. Louis, Missouri 2004, 86-151.
7. *Ferguson D. C.*: Euthyroid sick syndrome. Canine Pract. 1997, 22, 49-51.
8. *Freake H. C., Oppenheimer J. H.*: Thermogenesis and thyroid function. Annu. Rev. Nutr. 1995, 15, 263-291.
9. *Greco D. S.*: Congenital canine hypothyroidism. Canine Pract. 1997, 22, 23-24.
10. *Hoh Woo-Pil, Oh Tae-Ho*: Circadian variations of serum thyroxine, free thyroxine and 3,5,3' triiodothyronine concentrations in healthy dogs. J. Vet. Sci. 2006, 7, 25-29.
11. *Kantrowitz L. B., Peterson M. E., Melián C., Nichols R.*: Serum total thyroxine, total triiodothyroxine free thyroxine, and thyrotropin concentrations in dogs with nonthyroidal disease. JAVMA 2001, 219, 765-769.
12. *Kooistra H. S., Diaz-espineira M., Mol J. A., Van Den Brom W. E., Rijnberg A.*: Secretion pattern of thyroid-stimulating hormone in dogs during euthyroidism and hypothyroidism. Dom. Anim. Endocrinol. 2000, 18, 19-29.
13. *Kolevská J., Svoboda M., Brunclík V.*: Parallel Determinetion of Total Thyroxine and Thyrotropin Concentrations in Diagnosis of Primary Hypothyroidism in the Dog. Acta Vet. Brno 2002, 71, 61-67.
14. *Merchant S. R., Taboada J.*: Endocrinopaties: Thyroid and adrenal disorders. Vet. Clin. North. Am. Small. Anim. Pract. 1997, 27, 1285-1303.
15. *Nachreiner R. F., Fefsal K. R., Graham P. A., Hauptman J., Watson G. L.*: Prevalence of autoantibodies to thyroglobulin in dogs with nonthyroidal illness. AJVR 1998, 59, 951-955.
16. *Nelson R. W., Couto C. G.*: Disorders of the Thyroid Gland in Small Animal Internal Medicine. Mosby 2003, 691-709.
17. *Panciera D. L.*: It is possible to diagnose canine hypothyroidism? J. Small Anim. Pract. 1999, 40, 152-157.
18. *Panciera D. L.*: Thyroid-function testing: Is the future here? Vet. Medicine 1997, 92, 50-57.
19. *Patzl M., Most E.*: Determination of Autoantibodies to Thyroglobulin, Thyroxine and Triodothyronine in Canine Serum. J. Vet. Med. 2003, 50, 72-78.
20. *Peterson M. E., Melián C., Nichols R.*: Measurement of serum total thyroxine, triiodothyronine, free thyroxine, and thyrotropin concentrations for diagnosis of hypothyroidism in dogs. JAVMA 1997, 211, 1396-1402.
21. *Ramsey I.*: Diagnosis canine hypothyroidism. In Practice 1997, 9, 378-381.
22. *Scott-Moncrieff C. J., Nelson R. W., Bruner J. M., Williams D. A.*: Comparison of serum concentrations of thyroid-stimulating hormone in healthy dogs, hypothyroid dogs, and euthyroid dogs with concurrent disease. JAVMA 1998, 212, 387-391.
23. *Yong D. W.*: Antibodies to Thyroid Hormone and Thyroglobulin in Canine Lymphocytic Thyroiditis. Canine Pract. 1997, 22, 14-15.

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