The diagnosis of canine hypothyroidism and its differentiation from euthyroid sick syndrome still is a major diagnostic challenge. In this study, ultrasonography was shown to be an effective tool for the investigation of thyroid gland diseases. Healthy control dogs (n = 87), dogs with euthyroid sick syndrome (n = 26), thyroglobulin autoantibody-positive (TgAA-positive, n = 30) hypothyroid dogs, and TgAA-negative (n = 23) hypothyroid dogs were examined by thyroid ultrasonography. Maximal cross sectional area (MCSA), thyroid volume, and echogenicity were measured. Statistical analysis identified highly significant (P < .001) differences between euthyroid and hypothyroid dogs both in thyroid volume and in MCSA, whereas no significant differences in thyroid size were detected between healthy euthyroid dogs and dogs with euthyroid sick syndrome. In euthyroid and euthyroid sick dogs, parenchymal echotexture was homogeneous and hyperechoic, whereas relative thyroid echogenicity of both TgAA-positive and TgAA-negative hypothyroid dogs was significantly lower (P < .001). When using arbitrarily chosen cutoff values for relative thyroid volume, MCSA, and echogenicity, thyroid volume especially was found to have highly specific predictive value for canine hypothyroidism. In summary, the data reveal that thyroid sonography is an effective ancillary diagnostic tool to differentiate between canine hypothyroidism and euthyroid sick syndrome.

**Key words:** Canine hypothyroidism; Euthyroid sick syndrome; Thyroglobulin autoantibodies; Thyroiditis; Ultrasound.

Hypothyroidism is one of the most common endocrine disorders in dogs, but establishing the diagnosis can be a challenging task. Because of the widespread influences of thyroid hormones on cellular metabolism, clinical signs of hypothyroidism are quite variable and often vague, and they may resemble those of other disorders. Hypothyroidism can be associated with dermatological, neurological, reproductive, cardiovascular, hematological, and sometimes gastrointestinal signs. Therefore, a combination of several findings is required including history, clinical signs, and results of thyroid gland function tests. Commonly used screening protocols for evaluating thyroid gland function include diagnostic tests for baseline tT4 or fT4, canine thyroid stimulating hormone (cTSH), and thyroglobulin autoantibody (TgAA) serum concentrations. All diagnostic tests may have false negative as well as false positive results and are affected by several drugs (eg, glucocorticoids, anticonvulsants, nonsteroidal anti-inflammatory drugs, furosemide, some antibiotics, and tricyclic antidepressants) can markedly affect thyroid function and results of thyroid function tests.

Most canine hypothyroidism results from an atrophy of the thyroid gland caused by lymphocytic thyroiditis or idiopathic follicular atrophy. No diagnostic method yet has been established to measure these morphological changes in canine hypothyroidism. In contrast, in humans thyroid sonography routinely is used for the detection of morphological changes in the thyroid gland. Determination of thyroid size and thyroid echogenicity have been well documented as useful and valid diagnostic findings in autoimmune thyroid disease and thyroid dysfunction. Diffuse reduction in thyroid echogenicity is a sign of an active cytotoxic autoimmune process and is a valid predictor of autoimmune thyroiditis. Additionally, in humans a reduced thyroid volume is correlated with hypothyroidism.

Although the technique of a sonographic examination of the canine thyroid gland was introduced several years ago, it has not yet been evaluated as a diagnostic tool in canine hypothyroidism. In the present study, the applicability of ultrasound in the diagnosis of canine hypothyroidism was investigated. In particular, sonographic variables were developed that allowed differentiation between hypothyroidism and euthyroid sick syndrome.

**Materials and Methods**

**Dogs**

Thirty TgAA-positive hypothyroid dogs, 23 TgAA-negative hypothyroid dogs, and 26 dogs with euthyroid sick syndrome (ESS) presented to the Clinic for Small Animal Internal Medicine at the University of Munich between 2000 and 2003 were included in this study. Dogs with secondary hypothyroidism (2 dogs) or primary hypothyroidism caused by neoplastic destruction of the thyroid gland (3 dogs) were excluded. Eighty-seven healthy dogs presented to the clinic for routine examination and vaccination served as controls (Table 1). Clinically healthy dogs with low fT4 (<20 nM), low fT4 (<10 pM), or a positive TgAA titer were excluded from the control group (8 dogs). Animals were cared for according to the principles of the German law on protection of animal welfare.

**Determination of Thyroid Status**

Dogs were grouped as euthyroid, TgAA-positive hypothyroid, TgAA-negative hypothyroid, or euthyroid sick according to clinical signs, results of physical examination, CBC, serum biochemistry panel, and thyroid panel (fT4, fT4, TSH, TgAA) as described. Criteria for baseline tT4 or fT4, canine thyroid stimulating hormone (cTSH), and thyroglobulin autoantibody (TgAA) serum concentrations included diagnostic tests for baseline tT4 or fT4, canine thyroid stimulating hormone (cTSH), and thyroglobulin autoantibody (TgAA) serum concentrations. All diagnostic tests may have false negative as well as false positive results and are affected by several drugs (eg, glucocorticoids, anticonvulsants, nonsteroidal anti-inflammatory drugs, furosemide, some antibiotics, and tricyclic antidepressants) can markedly affect thyroid function and results of thyroid function tests.

**Key words:** Canine hypothyroidism; Euthyroid sick syndrome; Thyroglobulin autoantibodies; Thyroiditis; Ultrasound.
Table 1. Group characteristic of the 166 dogs in the study.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number</th>
<th>Median</th>
<th>Range</th>
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<th>Range</th>
</tr>
</thead>
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<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutered</td>
<td>10</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Female</td>
<td>19</td>
<td>4</td>
<td>2</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Neutered</td>
<td>26</td>
<td>6</td>
<td>2</td>
<td>10</td>
<td>4</td>
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</table>

Sonography

Ultrasoundography of the thyroid gland was performed in a quiet room with minimal restraint of the dogs. None of the dogs was sedated for examination. All dogs were examined in a sitting position. A small area (4 x 4 cm) caudal to the larynx was clipped, and coupling gel was applied. Sonographic examination of the thyroid glands was performed by 1 investigator (SR) with a 6-9 MHz linear transducer in large dogs (>25 kg) and a 7-13 MHz linear transducer in small dogs (<25 kg) by applying moderate probe pressure. At the time of sonographic examination, the sonographer did not know the category of the individual dogs.

Thyroid glands were scanned in longitudinal and transversal planes. The thyroid lobes were assessed by size, echogenicity, and homogeneity. To determine the volume of the thyroid lobes, the maximal length was measured in the longitudinal plane. In the next step, a transverse view of the thyroid gland was obtained by rotating the transducer 90 degrees and measuring the maximal width and height at the gland’s maximum cross sectional area. Thyroid lobe volume was calculated by means of the formula:

\[
\text{Volume (mL)} = \pi/6 \times \text{length (cm)} \times \text{width (cm)} \times \text{height (cm)}
\]

Total thyroid volume is given as the sum of left and right thyroid lobe volumes. In order to compare the quantitative variables among dogs of different sizes, thyroid volumes were related to metabolic body weight (BW⁰.⁷⁵). Additionally, the maximal cross sectional area (MCSA) of the thyroid lobes was measured as a second indicator of thyroid size. The MCSAs of both thyroid lobes were added and related to metabolic body weights. The reproducibility of the sonographic measurements was confirmed in 5 dogs that were examined 5 times. The value of the mean variation was 4.2%.

Because echogenicity can be altered by adjusting instrument parameters such as gain setting, echogenicity of the thyroid gland was compared with the echogenicity of the adjacent sternothyroid muscle as a reference tissue (Fig 1). To determine the echogenicity, the mean density (MD) of the MCSA of the thyroid lobes and the MD of the cross sectional area of the adjacent sternothyroid muscle were measured by means of image analyzing software. Relative echogenicity of the thyroid gland was calculated by means of the formula:

\[
\text{Relative echogenicity} = \text{MD (thyroid gland)} / \text{MD (sternothyroid muscle)}
\]

Muscle echogenicity can vary with the animal’s age or in relation to thyroid status. In this study, no statistically significant correlation was found between the echogenicity (MD) of the sternothyroid muscle and the age of the animals in the control group (r [Spearman] = 0.08, P = .452). Likewise, when comparing the mean echogenicity of the sternothyroid muscles of the control dogs and that of the hypothyroid dogs no statistically significant difference was found (Mann-Whitney U-test, P = .149).

To determine the diagnostic value of sonography in canine hypothyroidism, sensitivity, specificity, and accuracy were calculated for the different sonographic variables as suggested by Ferguson et al.³ Sensitivity was defined as the fraction of dogs that actually were hypothyroid that were labeled as hypothyroid by sonography. Specificity was defined as the fraction of dogs that actually were euthyroid that were labeled as euthyroid by sonography. Accuracy was defined as the fraction of all dogs that were neither falsely positive nor falsely negative.

Statistical Analysis

Commercially available software was used for statistical analysis. All values are presented as median and range. Differences among the 4 groups were analyzed by Mann-Whitney U-test. A P value <.05 was considered significant.

Results

In each of the 166 dogs included in this study, both thyroid lobes were identified by sonography, and complete measurements were performed in all dogs (Table 2). All dogs were grouped by the criteria described in the “Materials and Methods” section. Fifteen dogs were classified as ESS because of low serum concentration of tT4 (<20 nM) and tT4 (<10 pM), whereas in 9 ESS dogs tT4 concentrations were within the reference range. tTSH concentrations were within the reference range in 24 ESS dogs (92%). In both ESS dogs with increased cTSH concentrations the hormone concentrations returned to the normal range within 2 months after successful treatment of the concurrent illness. In contrast, in the group of TgAA-positive hypothyroid dogs 4 dogs (13%) had cTSH concentrations within the reference range, and in TgAA-negative hypothyroid dogs 11 dogs (48%) had cTSH concentrations within the reference range. Complete results of endocrine testing of each group (tT4, tT4, cTSH) are presented in Fig 1a-c. In 36 of the hypothyroid dogs and in 11 of the ESS dogs, TRH stimulation tests were performed. All of these hypothyroid dogs failed to respond to TRH, but also in 5 of the ESS dogs no (2 dogs) or only a low (3 dogs) response to TRH was observed, and the 4-hour tT4 concentration was <20 nM (Table 3). Finally, in all dogs hypothyroidism was confirmed by a positive response to thyroxine treatment.
Table 2. Median and range of the thyroid volume, relative thyroid volume, relative thyroid cross sectional area, and relative echogenicity in the 4 groups of dogs with different functional thyroid status.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Euthyroid Sick Dogs</th>
<th>TGAA-pos Hypothyroid Dogs</th>
<th>TGAA-neg Hypothyroid Dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid volume (ml)</td>
<td>Median</td>
<td>0.78</td>
<td>0.72</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0.08–2.2</td>
<td>0.21–1.52</td>
<td>0.08–1.22</td>
</tr>
<tr>
<td>Relative thyroid volume (ml/kg^{0.75})</td>
<td>Median</td>
<td>0.08</td>
<td>0.07</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0.03–0.13</td>
<td>0.04–0.12</td>
<td>0.01–0.12</td>
</tr>
<tr>
<td>Relative thyroid cross sectional area (mm^2/kg^{0.75})</td>
<td>Median</td>
<td>4.77</td>
<td>4.57</td>
<td>2.45</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>2.66–7.89</td>
<td>2.87–7.16</td>
<td>1.08–7.39</td>
</tr>
<tr>
<td>Relative echogenicity</td>
<td>Median</td>
<td>1.57</td>
<td>1.77</td>
<td>1.18</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>1.09–2.68</td>
<td>1.24–2.81</td>
<td>0.66–1.83</td>
</tr>
</tbody>
</table>

TGAA-pos, thyroglobulin autoantibody-positive; TGAA-neg, thyroglobulin autoantibody-negative.

Generally, the shape and MCSA of the thyroid lobes were different in euthyroid and ESS dogs as compared with both groups of hypothyroid dogs. In the majority of euthyroid and ESS dogs, the MCSA was triangular or polygonal (Fig 2), whereas the MCSA was oval in most hypothyroid dogs. In the longitudinal plane, the thyroid lobes were spindle shaped in all dogs of the 4 groups (Fig 3a–c).

Significant (P < .001) differences in the thyroid volume as well as in the MCSA were found between euthyroid dogs and hypothyroid dogs (Fig 4a), whereas no significant differences in thyroid size were detected between healthy euthyroid dogs and dogs with ESS. When comparing TGAA-positive and TGAA-negative hypothyroid dogs, no significant differences in thyroid size were identified (Fig 4a).

In euthyroid and ESS, thyroid parenchymal echotexture was homogeneous and hyperchoic as compared with the adjacent sternothyroid muscle (Figs 2, 3a). In contrast, the relative thyroid echogenicity of both TGAA-positive and TGAA-negative hypothyroid dogs was significantly lower (P < .001) as compared with euthyroid dogs (Fig 4c). In TGAA-positive hypothyroid dogs, thyroid echotexture was homogenously hypoechoic (Fig 3b), whereas the thyroid parenchyma in 10 TGAA-negative hypothyroid dogs showed a heterogeneous echotexture. In these dogs echotexture was characterized by a dark background interrupted by hyperechoic spots and lines (Fig 3c).
Table 3. Median and range of tT4 serum concentrations before as well as 2 and 4 hours after administration of 200 μg TRH IV in 36 hypothyroid dogs and 11 euthyroid sick dogs.

<table>
<thead>
<tr>
<th></th>
<th>tT4 (nM)</th>
<th>tT4 (nM)</th>
<th>tT4 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-TRH</td>
<td>2 Hours</td>
<td>Post-TRH</td>
</tr>
<tr>
<td>Hypothyroid dogs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>9.93</td>
<td>10.64</td>
<td>10.28</td>
</tr>
<tr>
<td>Range</td>
<td>1.53–19.1</td>
<td>1.59–20.1</td>
<td>1.54–19.4</td>
</tr>
<tr>
<td>Euthyroid sick dogs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>14.37</td>
<td>17.46</td>
<td>20.54</td>
</tr>
</tbody>
</table>

TRH, thyrotropin releasing hormone.

In order to further analyze the predictive value of ultrasound variables, cutoff values were chosen by trial and error to maximize sensitivity and specificity. The cutoff values were 0.05 mL/kg0.75 for relative thyroid volume, 3.3 mm²/kg0.75 for relative thyroid cross sectional area, and 1.4 for relative echogenicity. When a cutoff value for relative thyroid volume <0.05 mL/kg0.75 was used, sonography indicated hypothyroidism with a sensitivity of 81%, specificity of 96%, and accuracy of 91% (Table 4). With the relative thyroid cross sectional area with a cutoff value <3.3 mm²/kg0.75, the sensitivity was slightly reduced (77%), whereas specificity (96%) and accuracy (90%) were found to be comparably high. If a cutoff value of <1.4 for the relative echogenicity was used, the sensitivity for the detection of hypothyroidism was 75%, with a specificity of 80% and an accuracy of 78%. The highest sensitivity for the detection of hypothyroidism was found when a combination of relative thyroid volume and relative echogenicity was used. In this situation, hypothyroidism was predicted with a sensitivity of 98% if one or both of these variables were below the chosen cutoff values.

Discussion

Hypothyroidism is one of the most common endocrine diseases in dogs. Its diagnosis and differentiation between hypothyroidism and ESS still represent a major diagnostic challenge in dogs with low serum thyroid hormone concentrations.2,3 For example, the TRH stimulation test, which was used in place of the TSH stimulation test because of limited availability of TSH, was unsuitable to differentiate between ESS dogs and hypothyroid dogs. Although clinical and laboratory variables have been evaluated extensively as diagnostic tools, sonography has not been routinely used in this disease. This is the first report demonstrating that sonography is an effective diagnostic procedure that specifically enables discrimination between hypothyroidism and ESS in dogs.

Although earlier reports demonstrated that diagnostic ultrasound is an excellent method for the evaluation of canine thyroid gland size,46 examination of the thyroid gland by
Sonography in canine hypothyroidism has not yet been documented in the literature. First, this can be explained by the fact that no reference data on the thyroid size in healthy dogs are available. Secondly, the large range of thyroid size in dogs of different body weights makes it difficult to compare quantitative data among different breeds. To overcome these problems, thyroid sizes were compared by relating them to BW\(^{0.75}\). Both thyroid volume and MCSA were used as indicators of thyroid size in this study. Calculation of thyroid volume by the ellipsoid method (as applied in this study) is a well-established technique, but requires accurate sonographic technique and measurements to minimize interobserver variability. To address these problems, measurements were obtained by 1 single investigator (SR) in this study. However, results obtained with different ultrasonographers may be different. In future studies, the interobserver variability should be investigated. An additional possible error in calculation of the volume is the underestimation of the organ length due to difficulty in imaging the entire thyroid gland in the long axis scan plane. In contrast, measurement of the MCSA in the short axis plane is easy to perform and does not require additional calculation. With this technique, false data only will be obtained if an oblique scan plane is taken for measurement as previously reported for adrenal gland sonography.

Another possible source of error in calculating thyroid volume by means of a formula for an ellipsoid object is the variability in the cross sectional profile of the thyroid gland. However, former studies revealed a high correlation between the real volume of canine thyroid gland and the volumes calculated by the ellipsoid method. In the present study, the relative thyroid volume and the relative MCSA were found to be significantly lower in hypothyroid dogs as compared with euthyroid and ESS dogs. These results correlate with studies in humans that describe significantly reduced thyroid volumes in patients with hypothyroidism. Importantly, no differences were seen between euthyroid and ESS dogs. Therefore, ultrasonographic analysis of thyroid volume or MCSA allows differentiation between ESS and hypothyroid dogs.

To calculate the diagnostic value (ie, specificity, sensitivity, accuracy) of these thyroid variables, optimal cutoff values for thyroid volume and MCSA were determined. Relative thyroid volume and relative MCSA revealed high specificity (96%) and accuracy (91 and 90%), whereas sensitivity was found to be lower (81 and 77%). Therefore, both variables for thyroid size are excellently suited to con-
firm canine hypothyroidism. The diagnostic value of these thyroid variables is comparable to that of laboratory testing revealing low tT4 or fT4 by dialysis in combination with a high TSH concentration, which was reported to lead to the diagnosis of hypothyroidism in most dogs.26,27

Besides atrophy of the thyroid gland, the second most common pathological finding in canine hypothyroidism is atrophic autoimmune thyroiditis.12 In humans, determination of thyroid echogenicity has been well documented as a valid diagnostic test for the detection of autoimmune thyroiditis.16–20 Autoimmune thyroiditis sonographically is characterized by a hypoechoic echotexture of the thyroid parenchyma.16–22 Because this variable has not been evaluated in dogs, we sought to investigate changes in thyroid gland echogenicity in canine hypothyroidism.

Changes in echogenicity often are subtle, and differences in echo pattern may be difficult to detect. Furthermore, echogenicity can be altered by adjusting parameters such as gain setting. Therefore, perception of image density by the naked eye is unreliable.41 Two different methods generally are used to evaluate image density in an objective way. First, thyroid echogenicity can be characterized by standardized ultrasonography under defined operating conditions.20,42 However, standardized ultrasonography is difficult to achieve under practical conditions. Therefore, comparison of the echogenicity of the target structure with a reference tissue with the same conditions in 1 scan proved to be more useful in practical terms.31 In humans, relative echogenicity of the thyroid gland when compared with sternothyroid muscle was found to be useful to differentiate a healthy thyroid gland from a gland with thyroiditis.23 In dogs, the sternothyroid muscle (which lies near the thyroid gland) proved to be a more reliable tissue for comparison.

The results revealed that the relative echogenicity of the thyroid gland parenchyma was significantly (P < .001) lower in hypothyroid dogs as compared with euthyroid and ESS dogs. These results correlate with findings in humans in whom marked hypoechochogenicity of the thyroid gland implies an active autoimmune process and possibly a hypothyroid state.17,19,22 Interestingly, in 10 out of 23 TgAA-negative hypothyroid dogs an isoechoic or hyperechoic echotexture of the thyroid gland was found. In these dogs the echotexture was heterogeneous. We propose that this echotexture may reflect replacement of the thyroid tissue by fibrous and adipose tissue as described in dogs with noninflammatory atrophic hypothyroidism.43 In humans, a similar echotexture was described in the end stages of lymphocytic thyroiditis.44 Therefore, these observations further support the hypothesis that antibody-negative canine hypothyroidism represents a late stage of autoimmune thyroiditis.12,45–47

The variability in echotexture and echogenicity in thyroid glands of hypothyroid animals results in lower sensitivity, specificity, and accuracy of thyroid echogenicity as a diagnostic variable as compared with the thyroid size. Nevertheless, the results indicate that echogenicity may prove to be an effective variable for the detection of subclinical stages of canine hypothyroidism. When combining variables (relative thyroid volume and relative thyroid echogenicity) sonography is an excellent screening method with a high sensitivity (98%) for canine hypothyroidism. A comparable value is only achieved with the detection of low tT4 concentrations by the expensive and time-consuming equilibrium dialysis technique.35

In conclusion, thyroid sonography enabled us to establish a diagnosis in dogs with decreased thyroid hormone concentrations of unknown etiology. It is a reliable diagnostic tool to differentiate between canine hypothyroidism and ESS and an effective complement to established routine diagnostic tests. Thus, sonography facilitates the interpretation of laboratory test results by avoiding the erroneous diagnosis of canine hypothyroidism and resulting unwarranted treatment.

### Table 4

<table>
<thead>
<tr>
<th>Relative Thyroid Volume</th>
<th>Relative Cross Sectional Area</th>
<th>Relative Echogenicity</th>
<th>Low Relative Volume or Low Relative Echogenicity</th>
<th>Low MCSA or Low Relative Echogenicity</th>
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<tr>
<td>Sensitivity (%)</td>
<td>81</td>
<td>77</td>
<td>75</td>
<td>98</td>
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<tr>
<td>Specificity (%)</td>
<td>96</td>
<td>96</td>
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<tr>
<td>Accuracy (%)</td>
<td>91</td>
<td>90</td>
<td>78</td>
<td>84</td>
</tr>
</tbody>
</table>

### Footnotes

* Chemiluminescence Assay Elecs 1010 (previously validated for the dog*), Boehringer Mannheim Labdiagnostics, Mannheim, Germany
* rTSH–enzyme linked immunosorbent assay (previously validated for the dog*), Milenia Biotec GmbH, Bad Nauheim, Germany
* Enzyme linked immunosorbent assay, in house test previously validated for the dog*45–47
* TRH-Ferring, Kiel, Germany
* Siemens Sonoline Elegra ultrasound unit, Siemens AG, Erlangen, Germany
* Scion Image for Windows 4.02, Scion Corporation, Frederick, MD
* SPSS 11.5.1, SPSS Inc, Chicago, IL

### Acknowledgment

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ulin antibodies detected by enzyme-linked immunosorbent assay of

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