Assessment of left ventricular volumes by use of one-, two-, and three-dimensional echocardiography versus magnetic resonance imaging in healthy dogs

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Objective—To quantify left ventricle (LV) volumes by use of 1-D, 2-D, and 3-D echocardiography versus MRI in dogs.

Animals—10 healthy Beagles.

Procedures—During anesthesia, each dog underwent an echocardiographic examination via the Teichholz method, performed on the basis of standard M-mode frames (1-D); the monoplane Simpson method of disk (via 2-D loops); real-time triplane echocardiography (RTTPE) with a 3-D probe; and real-time 3-D echocardiography with a 3-D probe. Afterward, cardiac MRI was performed. Values for the LV end-diastolic volume (EDV), end-systolic volume (ESV), and ejection fraction (EF) were compared between each echocardiographic method and the reference method (cardiac MRI).

Results—No significant differences for EDV, ESV, and EF were detected between RTTPE and cardiac MRI. Excellent correlations (r = 0.97, 0.98, and 0.95 for EDV, ESV, and EF, respectively) were found between RTTPE and values for cardiac MRI. The other echocardiographic methods yielded values significantly different from cardiac MRI and results correlated less well with results of cardiac MRI for EDV, ESV, and EF. Use of the Teichholz method resulted in LV volume overestimation, whereas the Simpson method of disk and real-time 3-D echocardiography significantly underestimated LV volumes.

Conclusions and Clinical Relevance—Use of RTTPE yielded excellent correlations and nonsignificant differences with cardiac MRI and is a suitable method for routine veterinary cardiac examination. (Am J Vet Res 2013;74:1223–1230)

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A variety of canine congenital and acquired heart diseases may disturb hemodynamic balance and lead to volume overload and regurgitation with possible effects such as pulmonary edema, ascites, or sudden death from heart failure.1–3 Noninvasive evaluation of hemodynamic variables such as LV EDV, ESV, and EF is an essential part of routine veterinary cardiological examination.4 These variables have a strong influence on treatment decision making and estimation of prognosis.5 Therefore, it is necessary to obtain reliable results with a feasible method. Echocardiography is a technique frequently used to assess cardiac function and to measure ventricular volumes in veterinary medicine6 as well as in human medicine,7 whereas CMRI is the proposed noninvasive reference method for LV size and function measurements.8 Modern MRI systems are capable of rapid acquisition of imaging sequences because of stronger gradients and more homogeneous fields. Consequently, high-temporal-resolution images and dynamic cine records of the heart without any need of geometric assumptions enable precise analysis of cardiac anatomy and function.9 The main limitation of CMRI in veterinary medicine is the unavoidable use of anesthesia during the procedure.

In the past, 1-D echocardiography (M-mode) and 2-D echocardiography have been used to assess LV function and volume. However, M-mode overestimates the LV volume10 because of the accidental use of incorrect angled M-mode cuts and the dependence on geometric assumptions. Another ultrasonographic technique for LV volume calculation is the monoplane

ABBREVIATIONS

<table>
<thead>
<tr>
<th>CMRI</th>
<th>Cardiac MRI</th>
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<tr>
<td>EDV</td>
<td>End-diastolic volume</td>
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<td>EF</td>
<td>Ejection fraction</td>
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<td>ESV</td>
<td>End-systolic volume</td>
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<td>LV</td>
<td>Left ventricle</td>
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<td>RT3DE</td>
<td>Real-time 3-D echocardiography</td>
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<td>RTTPE</td>
<td>Real-time triplane echocardiography</td>
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<td>SMOD</td>
<td>Simpson method of disk</td>
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2-D SMOD. The SMOD evaluates the entire ventricle plane and is therefore supposed to be superior to the Teichholz method. Nevertheless, it may have limited accuracy because of use of foreshortened views in 2-D ultrasonographic loops as well as high dependence on experience and reliance on geometric modeling.

Presently, 3-D echocardiography is an additional option for cardiac volume estimation. Further development in 3-D ultrasonographic technique allows the use of modern matrix array probes, which include complex electronics and up to 3,000 individual elements, and enables multidirectional beam steering without the need of geometric assumption. Consequently, 3-D real-time imaging of the heart is possible with a substantial increase in accuracy and reproducibility in volume estimation over 2-D echocardiography. In human medicine, RT3DE has a high correlation with CMRI and is superior to 2-D echocardiography. Nevertheless, its widespread use in routine veterinary cardiac examination is limited because of high investment costs, time-consuming off-line analysis, and lack of veterinary studies indicating the superiority of RT3DE over 1-D and 2-D echocardiography for volume determination.

Additionally, use of RTTPE might avoid the limitations of M-mode and 2-D echocardiography. Simultaneous visualization of the LV in different projections during 1 cardiac cycle with high image quality allows simple analysis of LV volumes. Nuñofora et al reported high accuracy of RTTPE in volume estimation, compared with CMRI, in human medicine. To the authors’ knowledge, no study in veterinary medicine comparing RTTPE with CMRI has been reported.

The purpose of the study reported here was to evaluate LV volumes and function by use of the Teichholz method, SMOD, RTTPE, and RT3DE, compared with the gold-standard technique, CMRI, in healthy dogs. Our hypothesis was that determination of LV volumes and function by use of RTTPE and RT3DE would be more exact, compared with traditionally used echocardiographic measurements.

Materials and Methods

The study was approved by the Ethical Committee of the Lower Saxony State Office for Consumer Protection and Food Safety (33.9-42502-05-11A133). Ten healthy Beagles (2 females and 8 males; mean ± SD age, 6.8 ± 3.3 years, mean weight, 16.5 ± 1.8 kg) were enrolled in the study. The dogs were owned by the Small Animal Clinic, University of Veterinary Medicine Hannover. One day before the ECG and CMRI examination, all dogs underwent a detailed clinical examination, including blood pressure measurement, thoracic radiography, electrocardiography, CBC, serum biochemical analyses, and standard echocardiography, to determine healthiness and ensure normal LV diameters and function.

Echocardiography—The echocardiographic examination included M-mode echocardiography, 2-D echocardiography, RTTPE, and RT3DE and was performed by a single experienced investigator (SOH) with a commercial ultrasonographic scanner, equipped with a phased-array transducer, a matrix-array 3-V transducer, and ECG monitoring. The dogs were positioned alternately in right and left lateral recumbency on a raised table with a central opening designed for veterinary echocardiographic examinations. All images and loops were digitally stored and sent to a separate workstation equipped with commercially available software for off-line analysis. Each ultrasonographic method was analyzed by the same observer (JM) in random order. Each measurement was repeated 3 times, and mean values were calculated for further statistical analysis.

M-mode and 2-D echocardiography—M-mode and 2-D echocardiography images were performed and measured as recommended in the Guidelines of the American Association of Echocardiography by use of harmonic imaging with a phased-array transducer (2.0 to 4.5 MHz). The investigator optimized the visualization of the LV apex in the right parasternal long-axis view as well as in the left apical 4-chamber view, to constitute the maximal length of the LV and to avoid LV foreshortening. Penetration depth, image contrast, and image size were adjusted to obtain optimal visualization of the heart. Loops with a minimum of 3 consecutive cardiac cycles were stored.

Teichholz measurement—M-mode echocardiography of the LV was obtained from the right parasternal long-axis 4-chamber view. The LV-diameter was measured in end diastole and end systole; end diastole was defined as the beginning of the QRS complex and end systole as the maximal approximation of septum and LV posterior wall. End-diastolic and end-systolic diameters were measured by use of the leading edge-to-leading edge method (Figure 1). Measurements of 3 consecutive cardiac cycles were used to calculate the mean values. The EDV and ESV were calculated by use of the Teichholz equation, and EF was computed as follows: (EDV – ESV)/EDV × 100.

SMOD measurement—Monoplane SMOD measurement was performed in right parasternal long-axis view (SMOD-r) and in left apical 4-chamber view (SMOD-l) as described by Wess et al. End diastole was defined as the beginning of the QRS complex at the time of mitral valve closure, and end systole was defined as the minimal cavity area before mitral valve opening. The SMOD measurement required manual tracing along the endocardial border in the selected end-systolic and end-diastolic images from one side of the mitral annulus to the other, whereas papillary muscles and trabeculae were included in the LV volume calculation. The maximal length of the LV cavity was measured from the middle of the mitral annulus to the endocardial border of the LV apex. The EDV and ESV were automatically calculated by the ultrasonographic machine by use of the summation of the elliptical disks (usually 15 disks), whereas EF was calculated as described (Figure 2). The biplane SMOD could not be performed because an accurate 2-chamber view of the LV could not be obtained in all dogs.

3-D echocardiography—Three-dimensional echocardiography was performed immediately following
acquisition of 2-D echocardiography images. A matrix-array 3-V probe (2.5 to 3.6 MHz) in harmonic mode was used for both RTTPE and RT3D echocardiography. Scan depth and sector angle were optimized for maximal frame rate and best image resolution. Definition of end diastole and end systole was the same as for SMOD measurement.

RTTPE—The LV was simultaneously displayed in a quad screen in 3 planes. A fourth image in the quad screen revealed a dynamic 3-D reconstruction model of the LV, computed from the 3 planes (Figure 3). Loops from 3 consecutive cardiac cycles were acquired and digitally stored. For RTTPE analyses, LV adjustment in all 3 views was necessary to obtain the maximal LV length and to avoid foreshortening. Subsequently, manual tracing along the endocardial borders in all 3 apical planes was done in end diastole as well as in end systole. Papillary muscles and trabeculae were considered to be part of the ventricular cavity. The EDV and ESV were computed via surface triangulation and summation of all triangles by use of the divergence theorem. The EF was calculated as described.

RT3DE—In RT3DE mode, 4 pyramidal-shaped LV subvolumes from 4 consecutive cardiac cycles were acquired to cover the apical full volume in the 3-D data set with sufficient frame rates and consequently satisfactory image quality. The frame rate of the data sets was from 30 to 50 frames/s. The subvolumes were merged by ECG triggering, and the entire LV was displayed in different views in a quad screen. A semiautomatic analysis program for 4-D quantification of the LV was used to measure LV volumes and LV EF. First, gentle manual adjustment of the apical 4-chamber plane was necessary for correct positioning of the LV as described. Subsequently, end-systolic and end-diastolic frames were automatically identified. Placing one identification marker in the middle of the LV base and another at the LV apex, both in end diastole and end systole in all 3 apical long-axis views, was enough to start the automated endocardial border detection and to obtain a suitable, dynamic geometric model of the LV over 1 cardiac cycle (Figure 4). Manual correction was necessary in all cases to adjust the automatically generated border as close as possible to the endocardial border. The program computed EDV, ESV, and EF by use of a fitting geometric algorithm and created a dynamic cast of the LV cavity.

CMRI—All animals were shaved on the left side of the thorax for affixing the self-adhesive, MRI-fitting ECG electrodes in a right-angled array. Use of retrospective ECG gating was inevitable to reduce cardiac motion artifacts and obtain appropriate image acquisition. The dogs were positioned in a head-first supine position in the magnet bore. A combination of overlapping phased-array cardiac coils, with 1 coil unit lying under the dog and another above, was placed around the thorax at the level of the heart. All dogs received earplugs and were kept warm with heated gel cushions.

Cardiac MRI was performed by a single experienced investigator (PD) using a 3.0-T scanner. Before the start of each scanning sequence, heart rate was updated on the ECG gating system to reduce cardiac motion artifacts. All series were acquired during breath holding in expiration by turning the respirator off (maximum, 21 seconds). Sagittal, coronal, and transverse standard localizers were used to identify the ventricular long-axis, short-axis, horizontal long-axis, and 4-chamber planes according to a published method. These planes (each in end diastole and end expiration) enabled accurate planning of the transventricular short-axis sequence (22
slices, 4-mm slice thickness, and no gaps) perpendicular to the LV long axis from the LV apex to the mitral annulus. A sequence that featured ECG-gated fast imaging with a steady-state free precession dynamic gradient echo was used with the following acquisition parameters: field of view, 320 × 198 × 88 mm; repetition time, 4 milliseconds; echo time, 2 milliseconds; and pulse flip angle, 40°. All dynamic cine loops (30 frames/cardiac cycle) were digitally stored and sent to a special workstation for further analysis.

The EDV and ESV were measured off-line by 1 observer (JM) by use of commercial analysis software. Manual contouring of the LV epicardial and endocardial borders was done in each short-axis slice in end diastole as well as in end systole. Papillary muscles were included in the LV volume. Slices at the level of the heart base were included, if they were surrounded by at least 50% of the ventricular myocardium (Figure 5).21 The software calculated EDV and ESV independent of geometry by use of a modified SMOD equation. The EF was automatically computed as \((\text{EDV} - \text{ESV})/\text{EDV}\) × 100. Each measurement was repeated 3 times, and a mean value was calculated for statistical analysis.

Statistical analysis—Statistical analyses were performed with commercial analysis software. The LV volumes and EF values are expressed as mean ± SD. Normal distribution of the data was confirmed by use of the Shapiro-Wilk test. For each pair of values, limits of agreement and systematic errors were assessed by use of ANOVA. Comparisons between measurements were also performed by use of ANOVA. The strength of the relationship between each technique and CMRI reference values was evaluated by means of linear regression analysis with Pearson correlation coefficients. Bland-Altman analysis was performed to determine limits of agreement and systematic errors between the echo-cardiographic modalities and CMRI. A value of \(P < 0.05\) was considered significant.

Results

Examinations were successful in all 10 dogs. Mean ± SD heart rate during anesthesia was 87 ± 9 beats/min. Mean ± SD values of LV volumes and EF of each measurement technique were summarized (Table 1). Results of Pearson correlation analysis and the multiple comparisons between the different measurement techniques were determined (Table 2). Results of linear regression analysis were illustrated (Figure 6).

EDV and ESV—No significant difference was found between EDV and ESV values obtained via RTTPE and CMRI (Table 2) with an excellent correlation coefficient and low bias (Figure 7). The Teichholz method significantly overestimated LV EDV, whereas SMOD-r, SMOD-l, and RT3DE significantly underestimated LV EDV, compared with results obtained via CMRI. Results obtained with the Teichholz method, SMOD, and RT3DE correlated less well with CMRI results, with greater biases and wider limits of agreement.

EF—There were no significant differences between values of EF obtained via RTTPE versus CMRI (Table 2; Figure 7) or the Teichholz method versus CMRI. The
mean LV EF values obtained by use of SMOD-\textit{l}, SMOD-\textit{r}, and RT3DE were significantly higher than those obtained by use of CMRI. The EF values measured via RTTPE correlated well with the CMRI reference values. Weaker correlations were found between results obtained via CMRI versus the other techniques.

**Discussion**

Choosing the right treatment in dogs with cardiovascular disease requires a reliable evaluation of the current structure and function of the heart. Left-ventricular volume quantification is an important component in cardiac examination because volumes often increase during progressive diseases in which volume overload develops.\(^2^2\) The imprecision of conventional M-mode and 2-D echocardiography has been reported in several studies\(^4,18\) and was confirmed in the present study. Furthermore, the requirement for anesthesia in animals limits the routine use of CMRI, the technique that provides the most accurate results.\(^1^9\) Consequently, the present study was designed to determine the most precise echocardiographic technique, compared with CMRI, in anesthetized dogs. Three-dimensional echocardiography has been greatly improved over the past decade and has become an important tool in routine cardiologic examination in human medicine. Modern matrix-array transducer technology considerably improved image quality. Furthermore, novel semiautomatic analysis software reduced subjective effects and analyzing time.\(^2^3\)

The main finding of the present study in dogs was that LV volumes obtained via RTTPE were not significantly different from those obtained via CMRI, as also reported in a prior human study.\(^1^3\) Use of RTTPE was superior to the Teichholz method, SMOD, and RT3DE, as indicated by RTTPE's significant lower biases, compared with the other methods. Moreover, RTTPE is simpler and faster than RT3DE because it does not require acquisition of a full 3-D data set over several consecutive cardiac cycles. Consequently, RTTPE is feasible in dogs with respiratory sinus arrhythmia, as well. Use of RTTPE is also feasible in animals with high heart rates, although frame rate will decrease with increasing heart rate. The perceived main disadvantage is the need for manual tracing along the endocardial borders. Manual tracing is time-consuming and more subjective than automatic border detection.

The Teichholz method significantly overestimated LV volumes, compared with CMRI, as in a human study.\(^1^0\) In dogs, Tidholm et al\(^4\) also obtained higher values for LV volumes by use of the Teichholz method, compared with 2-D and 3-D echocardiographic techniques. The Teichholz method is based on the assumption that the LV is elliptical and calculates a 3-D volume from a 1-D measurement.

![Representative images of the LV (progressively numbered from 1 to 15) in a dog obtained by use of CMRI. Manual contouring of the LV epicardial and endocardial borders (yellow lines and green lines, respectively) was performed in each short-axis slice in end diastole. Papillary muscles were included in LV volume calculation.](image)

![Figure 5](image)

![Table 1](image)

**Table 1**—Mean ± SD values of LV EDV, ESV, and EF calculated by means of various echocardiographic methods in 10 dogs.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CMRI</th>
<th>Teichholz</th>
<th>SMOD-\textit{l}</th>
<th>SMOD-\textit{r}</th>
<th>RTTPE</th>
<th>RT3DE</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDV (mL)</td>
<td>37.14 ± 2.69</td>
<td>39.10 ± 3.35</td>
<td>34.00 ± 3.38</td>
<td>34.29 ± 3.45</td>
<td>36.78 ± 3.01</td>
<td>32.40 ± 4.15</td>
</tr>
<tr>
<td>ESV (mL)</td>
<td>19.68 ± 2.82</td>
<td>20.78 ± 2.81</td>
<td>16.99 ± 2.22</td>
<td>16.85 ± 3.18</td>
<td>19.23 ± 3.22</td>
<td>15.14 ± 2.30</td>
</tr>
<tr>
<td>EF (%)</td>
<td>47.22 ± 4.45</td>
<td>47.07 ± 3.72</td>
<td>50.27 ± 2.37</td>
<td>51.20 ± 5.69</td>
<td>47.91 ± 5.58</td>
<td>53.25 ± 2.80</td>
</tr>
</tbody>
</table>

\(l = \) Left apical 4-chamber view. \(r = \) Right parasternal long-axis view.

**Table 2**—Results of statistical comparisons (correlation and variance analyses) between various echocardiographic methods and CMRI used to calculate LV EDV, ESV, and EF in 10 dogs.

<table>
<thead>
<tr>
<th>Method</th>
<th>EDV</th>
<th>ESV</th>
<th>EF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Correlation</td>
<td>Variance analysis</td>
<td>Correlation</td>
</tr>
<tr>
<td></td>
<td>( r )</td>
<td>( P ) value</td>
<td>Bias</td>
</tr>
<tr>
<td>Teichholz</td>
<td>0.91 &lt; 0.01</td>
<td>-1.96 1.45 &lt; 0.01</td>
<td>0.98 &lt; 0.01</td>
</tr>
<tr>
<td>SMOD-\textit{l}</td>
<td>0.92 &lt; 0.01</td>
<td>3.14 1.40 &lt; 0.01</td>
<td>0.86 &lt; 0.01</td>
</tr>
<tr>
<td>SMOD-\textit{r}</td>
<td>0.92 &lt; 0.01</td>
<td>2.65 1.46 &lt; 0.01</td>
<td>0.93 &lt; 0.01</td>
</tr>
<tr>
<td>RTTPE</td>
<td>0.97 &lt; 0.01</td>
<td>0.36 0.74 0.16 0.98 &lt; 0.01</td>
<td>0.45 0.67 0.06</td>
</tr>
<tr>
<td>RT3DE</td>
<td>0.89 &lt; 0.01</td>
<td>4.74 2.13 &lt; 0.01</td>
<td>0.87 &lt; 0.01</td>
</tr>
</tbody>
</table>

See Table 1 for key.
Therefore, breed-dependent differences in ventricle geometry as well as changes in ventricular shape caused by cardiac diseases will cause under- or overestimation of real ventricular volumes. Moreover, incorrect cursor placement in M-mode leads to oblique visualization of the ventricle and subsequently to overestimation in volume calculation.

The significant underestimation of LV volumes by use of monoplane SMOD, compared with CMRI, is also described in human studies. No studies have been reported comparing the SMOD method with CMRI in animals. The monoplane and biplane SMOD methods are based on the assumption that the LV is a symmetric structure and are used to calculate a 3-D volume by measurement of areas in 1-D or 2-D planes. Consequently, SMOD accounts for breed-dependent varieties in ventricle anatomy or changes in ventricular shape, but adaption is not optimal because of 1 or 2 disregarded ventricle planes. This might cause under- or overestimation of real ventricular volumes. Furthermore, incorrect optimizing of the maximal long-axis leads to foreshortened views of the LV. The difficulty in attaining optimally angled planes of the LV is caused by the different thoracic shapes in dogs. Foreshortening leads to underestimation of the real ventricular volume and might be a reason for the lower values measured by use of SMOD. The biplane SMOD might yield more accurate results because it includes a second plane with the 2-chamber view, but it could not be used in the present study.
study because attainment of the 2-chamber view was not possible in all 10 dogs.

In the present study, RT3DE significantly underestimated LV volumes, compared with CMRI. To the authors’ knowledge, no studies have been reported in veterinary medicine comparing these 2 methods. Human studies also revealed significant underestimation of LV volumes by use of 3-D echocardiography, compared with CMRI.

Several factors might have contributed to the underestimation of LV by use of RT3DE in the study reported here. First, short-axis CMRI data were used and compared with echocardiographic long-axis measurements. Short-axis CMRI is currently the proposed reference technique for LV volume determination. In human medicine, Sugeng et al used radial long-axis CMRI data to minimize this limiting factor and decrease underestimation of RT3DE-derived volume data. Soliman et al suggested use of a combination of short-axis and long-axis CMRI analyses to improve correlation with RT3DE. Further studies in veterinary medicine are necessary to prove superiority of radial long-axis CMRI over short-axis CMRI.

Second, poor endocardial definition caused by insufficient image quality in RT3DE might lead to under- or overestimation of LV volumes. Jenkins et al determined that measurement accuracy increases by use of contrast RT3DE. No studies have been performed in veterinary medicine indicating an improvement in measurement accuracy by contrast enhancement.

Third, use of subvolumes to generate a complete data set for the ventricle is not an intrinsic component of RT3DE. Real-time 3-D echocardiography provides a depiction of the whole LV during 1 cardiac cycle, which results in dissatisfying image quality because of insufficient temporal resolution (< 12 frames/s), which already exists in anesthetized dogs with low heart rates. In the present study, ECG-triggered multiple-beat 3-D echocardiography imaging was used with a frame rate from 30 to 50 frames/s. The main limitation of subvolume-derived data sets in dogs is the high sensitivity to heart rate variability and breathing during acquisition, which leads to stitching artifacts. The impossibility of voluntary breath holding in animals during the waking state and the common occurrence of respiratory sinus arrhythmia in dogs may lead to respiratory motion artifacts and gating limitations, which make RT3DE unfeasible in those patients.

Fourth, in the present study, the image of the LV was generated out of 4 subvolumes during RT3DE. Left-ventricle cutting is possible up to 7 pieces, which increases temporal resolution but makes the LV visualization more susceptible to additional motion artifacts.

Fifth, the 4-D probe of the ultrasonographic scanner is quite oversized for the narrow intercostal space in small dogs, which causes artifacts and reduces the acoustic window. Moreover, the low-frequency spectrum (2.5 to 3.6 MHz) is not optimal for evaluation of small hearts. This may lead to impaired image quality and, consequently, inaccurate automatic border detection, which then requires subjective and time-consuming manual border delineation.

Results of the study reported here indicated that triplanar echocardiography is an accurate technique for assessment of LV volumes and EF by use of CMRI as the reference standard, triplanar echocardiography was determined to be superior to conventional 1-D and 2-D echocardiographic methods. Data acquisition via RTTPE is rapid and easy to perform. Consequently, RTTPE might be useful for a clinical setting in dogs with valvular heart disease or other cardiac disorders associated with volume overload in the LV. Serial follow-up use of RTTPE might therefore be helpful in determining the progress of disease and the efficacy of pharmacological interventions. However, further studies are necessary to examine the modern RTTPE technique in dogs with heart disease. The use of RT3DE in routine veterinary clinical practice is still uncommon because of the high cost of the ultrasonographic machine, time-consuming off-line analyses, the need for different probes for dogs with different weights and shapes, and the lack of practicability in dogs with high heart rates or arrhythmia.

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