Pulmonary hypertension has been recognized as a clinical problem for many years in veterinary medicine. Routine accurate clinical diagnosis of pulmonary hypertension in dogs specifically has been markedly enhanced by the widespread use of echocardiography.\(^1\) However, systematic assessment of function of the right chambers of the heart is not uniformly carried out. This lack of consistency is due in part to the high amount of attention given to evaluation of the left chambers of the heart, a lack of familiarity with ultrasonographic techniques available for imaging the right chambers, and a paucity of ultrasonographic studies providing reference intervals for size and function of the right chambers.\(^2\) To date, reference intervals and repeatability of right-chamber heart function indices for echocardiographic evaluation, such as peak systolic tricuspid annulus velocity, tricuspid annulus plane systolic excursion, and systolic longitudinal right ventricular strain, have been reported.\(^3\)\(^-\)\(^6\) In addition, right-chamber heart function tests involving echocardiography have been used in clinical settings for dogs.\(^3\)\(^-\)\(^6\)\(^7\) These indices, however, provide an assess-

### ABBREVIATIONS

- CI: Confidence interval
- CV: Coefficient of variation
- DPD: Dual pulsed-wave Doppler
- ICC: Intraclass correlation coefficient
- ICT: Isovolumic contraction time
- IRT: Isovolumic relaxation time
- LS: Least squares
- RTX: Right ventricular Tei index
- \(\text{RTX}_{\text{DPD}}\): Right ventricular Tei index derived from DPD echocardiography
- \(\text{RTX}_{\text{TD}}\): Right ventricular Tei index derived from tissue Doppler echocardiography
- \(\text{RTX}_{\text{PD}}\): Right ventricular Tei index derived from conventional pulsed-wave Doppler echocardiography

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**OBJECTIVE**

To evaluate repeatability and reproducibility of right ventricular Tei index (RTX) values derived from dual pulsed-wave Doppler, conventional pulsed-wave Doppler, and tissue Doppler echocardiography and to investigate relationships and repeatability among the 3 methods in healthy dogs.

**ANIMALS**

6 healthy adult Beagles.

**PROCEDURE**

Echocardiography was performed on each dog on different days for 2 weeks (3 times/d) by 2 echocardiographers. Intraobserver within- and between-day and interobserver coefficients of variation (CVs) and intraclass correlation coefficients (ICCs) for RTXs derived from dual pulse-waved Doppler (\(\text{RTX}_{\text{DPD}}\)), conventional pulsed-wave Doppler (\(\text{RTX}_{\text{PD}}\)), and tissue Doppler (\(\text{RTX}_{\text{TD}}\)) methods were determined. Degrees of agreement among RTX values derived from the 3 methods were assessed by modified Bland-Altman analysis.

**RESULTS**

Least squares mean (95% confidence interval) \(\text{RTX}_{\text{TD}}\) was 0.50 (0.46 to 0.54), which was significantly higher than that for \(\text{RTX}_{\text{DPD}}\) (0.27 [0.23 to 0.31]) and \(\text{RTX}_{\text{PD}}\) (0.25 [0.21 to 0.29]). Agreement between \(\text{RTX}_{\text{DPD}}\) and \(\text{RTX}_{\text{PD}}\) was good (bias [mean difference], 0.04 [95% confidence interval, –0.03 to 0.10]).

The \(\text{RTX}_{\text{DPD}}\) had high within-day (CV, 6.1; ICC, 0.77) and interobserver (CV, 3.5; ICC, 0.83) repeatability, but between-day repeatability was not high. The \(\text{RTX}_{\text{TD}}\) had high within-day repeatability (CV, 6.0; ICC, 0.80), but between-day and interobserver repeatability were not high. Within-day, between-day, and interobserver repeatability of \(\text{RTX}_{\text{PD}}\) were not high.

**CONCLUSIONS AND CLINICAL RELEVANCE**

\(\text{RTX}_{\text{DPD}}\) measurement was a repeatable and reproducible method of cardiac evaluation in healthy dogs. The \(\text{RTX}_{\text{TD}}\) values were significantly higher than the \(\text{RTX}_{\text{DPD}}\) and \(\text{RTX}_{\text{PD}}\) values; therefore, RTX values derived from different echocardiographic methods should be interpreted with caution. (Am J Vet Res 2016;77:715–720)
Dogs

Six laboratory Beagles (2 females and 4 males) between 1 and 3 years of age and weighing between 9.5 and 13.0 kg were used in this study. All dogs were determined to be healthy with unremarkable heart anatomy and myocardial function on the basis of complete physical, ECG, and standard echocardiographic examinations (including M-mode, pulsed-wave Doppler, and color flow Doppler imaging). All procedures were approved by the Laboratory Animal Experimentation Committee, Graduate School of Veterinary Medicine, Hokkaido University.

Echocardiographic measurements

Conventional echocardiographic examinations were performed by 1 echocardiographer (KN), who used an ultrasonographic machine equipped with a sector probe (3 to 7 MHz). Unsedated dogs were manually restrained for evaluation in left and right lateral recumbency. An ECG trace (lead II) was recorded simultaneously with echocardiographic imaging and automatically measured heart rate. All dogs were confirmed healthy by echocardiographic examination.

The RTX was derived from DPD, conventional pulsed-wave Doppler, and tissue Doppler findings by 2 echocardiographers (KN and TM). For this process, RTX was defined as the sum of the ICT and IRT, divided by ejection time (Figure 1). For each RTX, mean values of 3 separate cardiac cycles were used to assess repeatability. Each RTX value was calculated after image acquisition.

To calculate RTX_{DPD}, tricuspid inflow and pulmonary artery flow were measured simultaneously by means of DPD echocardiography with a left parasternal short-axis view; and ICT and IRT were derived by subtracting the ejection time from the amount of time that elapsed between cessation of the tricuspid valve A wave to the onset of the tricuspid valve E wave in 1 image (Figure 1). Ejection time was measured from the beginning of one to the beginning of the next pulmonary arterial spectrum.

To calculate RTX_{TD}, tricuspid valve inflow was measured from a left parasternal short-axis view. Next, pulmonary arterial flow was measured from a left parasternal short-axis view. Finally, ICT and IRT were derived by subtracting ejection time from the amount of time that elapsed between cessation of the tricuspid valve A wave (late diastolic flow) to the onset of the tricuspid valve E wave (early diastolic flow) in 2 separate images (Figure 1). No attempt was made to match R-R intervals for the in- and outflow signals because, in the authors’ experience, it is difficult to match R-R intervals in clinical settings.

To calculate RTX_{TD}, the tricuspid valve peak systolic annular velocity, peak early diastolic velocity, and late diastolic velocity were determined by tissue Doppler echocardiography with an apical 4-chamber view. Then, ICT and IRT were derived by subtracting the duration of the late systolic annular velocity wave for the tricuspid valve from the time that elapsed between the end of the late diastolic velocity wave and onset of the early diastolic velocity wave for the same valve on the basis of tissue Doppler recordings (Figure 1).

Statistical analysis

Power calculations for sample size determination were made on the basis of data from a previous study. Presuming a similar intraobserver within-day, intraobserver between-day, and interobserver ICC, it was estimated that a sample size of 6 dogs would be required to provide a power of 90% to detect an ICC of 0.75, with the null hypothesis that the ICC would be 0, an α value of 0.05, and 3 measurements/technique/dog.

Statistical analysis programs were used to develop a linear mixed model, with measurement time (1 to 9 times), method (DPD, tissue Doppler, and pulsed-
wave Doppler techniques), and their interaction as categorical fixed effects and dog identity as a random effect. The F test was performed to assess the effect of measurement time and method on RTX. Multiple comparisons were made by obtaining the LS mean of 1 observer’s (KN) measurements and applying the Tukey honest significant difference test to assess differences among methods. The all-pairs Tukey test allows significance tests of all combinations of pairs, and the resulting honest significant difference intervals are greater than those provided with the Student pairwise t test for least significant differences.

The following linear model was used for within- and between-day and interobserver variability analyses:

\[
Y_{ijkl} = \mu + \text{observer}_i + \text{day}_j + \text{dog}_k + (\text{observer} \times \text{dog})_{ik} + (\text{day} \times \text{dog})_{jk} + \varepsilon_{ijkl}
\]

where \(Y_{ijkl}\) was the first value measured for dog \(k\) on day \(j\) by observer \(i\), \(\mu\) was the general mean, observer was the differential effect (considered as fixed) of observer \(i\), \(\text{dog}_k\) was the differential effect of dog \(k\), (observer \times dog) \(_{ik}\) represented the interaction between the observer and dog, (day \times dog) \(_{jk}\) represented the in-

**Figure 1**—Echocardiographic images illustrating a technique used to measure RTX in dogs by means of DPD (A), pulsed-wave Doppler (B and C), and tissue Doppler (D) techniques. A—The upper waveform is tricuspid inflow, and the lower waveform is pulmonary artery flow. B—Tricuspid inflow is shown. C—Pulmonary artery flow is shown. D—Tricuspid valve annular velocity is shown. The simultaneously recorded ECG appears at the top. For all calculations, RTX = \((a - b)/b\). \(a\) = Interval from tricuspid valve closure to opening (pulsed-wave Doppler recordings) or the end of the \(A'\) wave to the beginning of the \(E'\) wave (tissue Doppler recordings). \(A\) = Tricuspid valve late diastolic flow. \(A'\) = Tricuspid valve late diastolic velocity. \(b\) = Ejection time or \(S'\) duration. \(E\) = Tricuspid valve early diastolic flow. \(E'\) = Tricuspid valve early diastolic annular velocity. \(S'\) = Tricuspid valve systolic annular velocity.
The interaction between day and dog, and $\varepsilon_{ijkl}$ was the model error. The SD of within-day variability was estimated as the residual SD of the model, SD of between-day variability as the SD of the differential effect of day, and SD of interobserver variability as the SD of the differential effect of observer. The corresponding CVs were determined by dividing each SD by the mean.

The intraobserver within-day ICC was determined from data generated by the same observer (KN); this echocardiographer evaluated 6 dogs 3 times during the same day. The intraobserver between-day ICC was determined from data generated by 1 blinded observer (KN); on each of 3 days, this echocardiographer made 3 evaluations of the 6 dogs. The interobserver ICC was determined from data generated by 2 blinded observers (KN and TM) on the same day; these echocardiographers evaluated 6 dogs 3 times during the same day. Agreement was considered high when the CV was <20% and the ICC was >0.75.

Differences among measurements derived from the 3 methods were evaluated by use of Bland-Altman analysis, with modification for repeated measures as described elsewhere. Mean differences (bias) and 95% CIs were calculated. Differences among methods were considered significant when the 95% CI did not contain 0. Values of $P < 0.05$ were considered significant. Results are summarized as LS mean (95% CI).

### Results

Least squares means for variables associated with RTX as measured by 1 observer using the 3 echocardiographic methods were summarized (Table 1). The RTX\textsubscript{TD} values were significantly higher than the RTX\textsubscript{DPD} and RTX\textsubscript{PD} values. In contrast, RTX\textsubscript{DPD} did not differ significantly from RTX\textsubscript{PD}. Intervals between tricuspid valve closure and opening and isovolumic time (sum of ICT and IRT) derived from the tissue Doppler method were longer than respective values derived from the DPD and pulsed-wave Doppler methods. No difference in LS mean heart rate was identified among the 3 methods.

Bland-Altman analysis revealed that RTX\textsubscript{TD} values were significantly higher than RTX\textsubscript{DPD} and RTX\textsubscript{PD}.

### Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>DPD</th>
<th>Tissue Doppler</th>
<th>Pulsed-wave Doppler</th>
</tr>
</thead>
<tbody>
<tr>
<td>RTX</td>
<td>0.27 (0.23–0.31)\textsuperscript{a}</td>
<td>0.50 (0.46–0.54)\textsuperscript{b}</td>
<td>0.25 (0.21–0.29)\textsuperscript{a}</td>
</tr>
<tr>
<td>TCO (ms)</td>
<td>252 (236–268)\textsuperscript{a}</td>
<td>286 (270–292)\textsuperscript{b}</td>
<td>245 (229–261)\textsuperscript{a}</td>
</tr>
<tr>
<td>Ejection time (ms)</td>
<td>199 (189–209)\textsuperscript{a}</td>
<td>191 (181–201)\textsuperscript{b}</td>
<td>196 (186–206)\textsuperscript{a}</td>
</tr>
<tr>
<td>ICT + IRT (ms)</td>
<td>53 (43–63)\textsuperscript{a}</td>
<td>95 (85–105)\textsuperscript{b}</td>
<td>49 (39–59)\textsuperscript{a}</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>93 (77–109)\textsuperscript{a}</td>
<td>91 (75–107)\textsuperscript{b}</td>
<td>94 (78–110)\textsuperscript{a}</td>
</tr>
</tbody>
</table>

TCO = Interval between tricuspid valve closure and opening.

\textsuperscript{a,b}Values in the same row with different superscript letters are significantly ($P < 0.05$; Tukey test) different.

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Differences among measurements derived from the 3 methods were evaluated by use of Bland-Altman analysis, with modification for repeated measures as described elsewhere. Mean differences (bias) and 95% CIs were calculated. Differences among methods were considered significant when the 95% CI did not contain 0. Values of $P < 0.05$ were considered significant. Results are summarized as LS mean (95% CI).
values (Figure 2). However, agreement was good between RTX_{TD} and RTX_{pD}.

Intraobserver within- and between-day and interobserver CVs and ICCs of RTX derived from the 3 methods were summarized (Table 2). The RTX_{pD} had high (low CV and high ICC) within-day and interobserver repeatability, but between-day repeatability was not high. The RTX_{TD} had high within-day repeatability, but between-day and interobserver repeatability were not high. The RTX_{pD} lacked high within- and between-day repeatability and interobserver repeatability.

**Discussion**

In the present study, within-day and interobserver repeatability of RTX_{pD} measurements were high in a small number of healthy Beagles. Findings provided the first description of repeatability of RTX_{pD}, RTX_{pD}, and RTX_{TD} measurements in dogs and were consistent with values reported for humans. The DPD method allows simultaneous recording of Doppler signals at 2 points during the same cardiac cycle; therefore, RTX_{pD} measurement is not influenced by heart rate fluctuation. Because respiratory arrhythmia is common in dogs, measurements obtained with the DPD method versus other methods are suggested to be more accurate in that species.

Intraobserver within- and between-day and interobserver repeatability and reproducibility of RTX_{pD} measurement were low in the present study. This is in disagreement with the results of a previous study, in which the between-day CV for RTX_{pD} measurement in 55 healthy dogs was 15.3%. This difference may be related to the number of measurements, in that 3 cardiac cycles were used in the present study versus 20 cycles in the other study.

High intraobserver and low interobserver repeatability of RTX_{TD} measurement were obtained in the present study. To date, repeatability and reproducibility of RTX_{TD} measurement in dogs has lacked adequate evaluation. For humans, high and low repeatability and reproducibility of RTX_{TD} measurement have been reported. The RTX_{TD} can also be measured in a single cardiac cycle; therefore, it is not influenced by heart rate fluctuations. Low interobserver repeatability in humans and the dogs of the present study may be attributable in part to fact that limits of different intervals for tissue Doppler echocardiography are often poorly defined and may be too sensitive to mild changes, such as hemodynamic shifts or slight differences in the obtained images.

The RTX_{TD} values were higher than the RTX_{pD} and RTX_{pD} values of the dogs of the present study. This finding was consistent with findings of previous studies involving humans and dogs. The higher RTX_{TD} was mainly attributable to the longer interval between tricuspid valve closure and opening and iso-volumic time derived from the tissue Doppler method, compared with values obtained with DPD and pulsed-wave Doppler methods. The reason for differences between RTX_{TD} and other RTX measurements may have been related to differences in methods used and measurement sites. The RTX_{TD} is measured by use of intervals based on myocardial motion, whereas the RTX_{pD} and RTX_{pD} are measured by use of intervals based on blood flow. Moreover, RTX_{TD} is measured only at the right ventricular inlet portion, in contrast to RTX_{pD} and RTX_{pD}, which are measured at both the right ventricular inlet and outlet portions. Therefore, RTX_{TD} may be unrelated to the overall right ventricular function. It is important to consider that RTX_{TD} had higher reference values than did RTX_{pD} and RTX_{pD}; therefore, Tei indexes should not be used interchangeably.

In humans with right ventricular overload, RTX_{pD} is a better predictor of exercise capacity than is RTX_{TD} and RTX_{pD}. Therefore, in dogs with right ventricular overload, as occurs with pulmonary hypertension, RTX_{pD} may also be a better predictor of right heart dysfunction. Additional studies are needed to validate the clinical usefulness of RTX_{pD} measurement in dogs with right heart dysfunction.

The present study had several limitations. First, a small number of healthy laboratory Beagles was used; therefore, caution should be exercised when attempting to extrapolate the repeatability and reproducibility data to dogs with right heart dysfunction. Indeed, in humans, the degree of disagreement among RTX values in patients with heart disease is higher than that in healthy subjects. Second, no reference standard of the right ventricular function, such as cardiac catheterization, was evaluated in the present study. Therefore, we could not assess which of the 3 methods for RTX measurement was superior. Additional studies are needed to validate the correlation between RTX and right ventricular function obtained by cardiac catheterization and other noninvasive echocardiographic

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**Table 2**—Within- and between-day (1 observer) and interobserver (2 observers) CVs and ICCs for 3 echocardiographic methods of RTX measurement in healthy adult Beagles (n = 6) performed 3 times/d for 3 days.

<table>
<thead>
<tr>
<th>Method</th>
<th>Within-day</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CV (%)</td>
<td>ICC</td>
<td>CV (%)</td>
<td>ICC</td>
<td>CV (%)</td>
</tr>
<tr>
<td>DPD</td>
<td>6.1</td>
<td>0.77</td>
<td>8.4</td>
<td>0.73</td>
<td>3.5</td>
</tr>
<tr>
<td>Tissue Doppler</td>
<td>6.0</td>
<td>0.80</td>
<td>7.7</td>
<td>0.63</td>
<td>24.6</td>
</tr>
<tr>
<td>Pulsed-wave Doppler</td>
<td>20.7</td>
<td>0.62</td>
<td>20.7</td>
<td>0.35</td>
<td>19.1</td>
</tr>
</tbody>
</table>

Agreement was considered high when the CV was < 20% and the ICC was > 0.75.
indices, such as tricuspid valve annular plane systolic excursion or fractional area change. Third, DPD echocardiography is a novel application of ultrasonography that is available on only few ultrasonographic systems, so the usefulness of RTX_{DPD} measurement may be limited in clinical settings.

The study reported here revealed that RTX_{DPD} measurement was a feasible and reliable method for evaluation of cardiac function in a small number of healthy dogs. The RTX_{DPD} values were not significantly different from the RTX_{TD} values; however, RTX_{TD} values were significantly higher than RTX_{DPD} and RTX_{PD} values. Therefore, RTX values derived from different methods should be interpreted with caution and not used interchangeably because values differ with each method. Investigations involving dogs with heart disease are warranted to determine the clinical applicability of RTX_{DPD} measurement.

Acknowledgments

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Footnotes

a. HI VISON Preirus, Hitachi Medical Corp, Chiba, Japan.
b. EUP-S52, Hitachi Medical Corp, Chiba, Japan.
c. JMP version 8.0, SAS Institute Inc, Cary, NC.
d. SPSS, version 21, SPSS Inc, Chicago, Ill.

References