

# Effects of animal position and number of repeated measurements on selected two-dimensional and M-mode echocardiographic variables in healthy dogs

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**Objective**—To evaluate the effects of positioning and number of repeated measurements on intra- and interobserver variability of echocardiographic measurements in dogs.

**Design**—Prospective study.

**Animals**—4 healthy dogs.

**Procedure**—Each observer performed 24 examinations, separately assessing each dog 6 nonconsecutive times (3 times with the dog in lateral recumbency and 3 with the dog in a standing position). Variables evaluated included M-mode measurements of left ventricular end-diastolic and left ventricular end-systolic diameters, left ventricular free-wall thickness in diastole and systole, interventricular septal thickness in diastole and systole, left ventricular shortening fraction, and 2-dimensional measurements of the left atrial diameter-to-aortic diameter ratio.

**Results**—All coefficients of variation (range, 3.4% to 26.6%) were similar between operators and positions and were < 15% for 27 of 32 values. For both operators, repeatability of the measurements was better for left ventricular end-systolic diameter, left ventricular free-wall thickness in diastole, left ventricular free-wall thickness in systole, and the left atrial diameter-to-aortic diameter in the standing position, and similar for both positions for shortening fraction and left ventricular end-diastolic diameter. No effect of cardiac cycle was observed.

**Conclusions and Clinical Relevance**—Within-day variability of conventional echocardiography performed with the dog in the standing position was at least as good as that obtained with the dog in lateral recumbency for most measured variables. Single measurements of each variable may be sufficient for trained observers examining dogs that do not have an arrhythmia. The standing position should be used, particularly for stressed or dyspneic dogs. (*J Am Vet Med Assoc* 2005;227:743–747)

Present recommendations for performing complete echocardiographic studies in dogs include obtaining images in 2-dimensional (2-D), M-mode, and Doppler modes. Even in large-breed dogs, patients are often positioned in lateral recumbency to decrease interference from the lungs.<sup>1</sup> Practical reasons for per-

forming examinations with the subject in recumbency include ease of restraint by 1 handler, to facilitate collection of Doppler data, and variation in the heights of standing dogs. However, performing the examinations with the subject in lateral recumbency has disadvantages—it increases stress and may influence echocardiographic results in nervous dogs. Moreover, some animals cannot tolerate a recumbent position because of discomfort or dyspnea. Although positioning dogs in lateral recumbency is thought to ensure thorough and repeatable examinations,<sup>2</sup> determination of the within-day and between-day variability of conventional echocardiographic variables in standing cats<sup>3</sup> and dogs<sup>4</sup> indicates that both repeatability and reproducibility are good if the observers are suitably trained.

Another recommendation<sup>1</sup> for performing echocardiographic studies is to record 3 to 5 consecutive cardiac cycles and calculate the mean of the measurements for each variable to negate the effects of respiration and changes in ventricular filling associated with sinus arrhythmia. However, the relevance of this time-consuming procedure has not been evaluated.

The purposes of this study were to compare the values and variability of echocardiographic measurements obtained in laterally recumbent versus standing dogs and to assess the effect of the number of measurements on the final value of each variable.

## Materials and Methods

**Dogs**—The procedures in this study were in accordance with the *Guide for the Care and Use of Laboratory Animals*<sup>5</sup> and approved by the Animal Use and Care Committee of the National Veterinary School of Alfort. Four dogs were used and included 2 sexually intact female Beagles (age, 1.8 and 5.5 years; body weight, 7.4 and 9.8 kg), 1 male Labrador Retriever (14 months; 27 kg), and 1 male Golden Retriever (13 months; 28.7 kg). The dogs were considered healthy on the basis of results of a physical examination, an ECG, and an indirect arterial blood pressure measurement, all performed before inclusion in the study.

**Echocardiography**—Transthoracic echocardiography was performed with a 2.2- to 3.5-MHz phased-array transducer,<sup>6</sup> with continuous ECG monitoring. All examinations were performed in conscious dogs that were gently restrained in a standing position or in right lateral recumbency. Ventricular measurements were obtained by use of 2-D–guided M-mode in

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the right parasternal ventricular short-axis view<sup>6</sup> according to the recommendations of the American Society of Echocardiography.<sup>7</sup> Left ventricular end-diastolic diameter (LVDD), left ventricular end-systolic diameter (LVSD), left ventricular free-wall thickness in diastole (LVFWD), left ventricular free-wall thickness in systole (LVFWS), interventricular septal thickness in diastole (IVSD), and interventricular septal thickness in systole (IVSS) were measured. The left ventricular shortening fraction (SF) was calculated. Measurements of the aortic diameter (Ao) and the left atrial (LA) dimension were performed by means of a 2-D echocardiographic method,<sup>8</sup> with a short-axis right-sided parasternal view obtained at the level of the aortic valve, where the commissures of the cusps were visualized during early diastole. The internal short-axis diameter of the aorta was measured along the commissure between the noncoronary and left coronary aortic valve cusps. The LA was measured from the same frame in a line extending from and parallel to the commissure between the noncoronary and left coronary aortic valve cusps. The LA/Ao ratio was calculated. All measurements were made directly from the monitor freeze-frame image and were timed. For each M-mode echocardiographic examination, 5 measurements of each variable were obtained in 5 consecutive cardiac cycles. The heart rate was determined from the ECG.

**Observers**—Examinations were performed by 2 diplomates of the European College of Veterinary Internal Medicine (cardiology) with > 15 years of expertise in echocardiography. Observer 1 (AT) was accustomed to performing echocardiographic examinations with dogs in lateral recumbency, whereas observer 2 (VC) was accustomed to performing the examinations in standing dogs. Observers 1 and 2 trained each other in use of the alternative position for 2 consecutive weeks prior to performing the examinations.

**Within-day intraobserver variability**—Forty-eight echocardiographic examinations were performed on the same day; each observer performed 24 examinations, separately assessing each dog 6 times (3 examinations with the dog in lateral recumbency and 3 with the dog in a standing position). No dog was examined 2 times in succession. All echocardiographic examinations were randomized and blinded. The observer performed the measurements with calipers but was unable to see the values on the screen. A third person was in charge of data collection.

**Statistical analyses**—A software program<sup>b</sup> was used to perform the analyses. A general linear model was used as described<sup>4,9</sup> for assessment of repeatability of results. The following model was used:

$$Y_{ijkl} = \mu + \text{dog}_i + \text{position}_j + \text{operator}_k + \text{cardiac cycle}_l + (\text{dog} \times \text{position})_{ij} + (\text{dog} \times \text{operator})_{ik} + (\text{position} \times \text{operator})_{jk} + e_{ijkl}$$

Table 1—Effects of dog position and operator on left ventricular end-diastolic diameter (LVDD), left ventricular end-systolic diameter (LVSD), left ventricular free-wall thickness in diastole (LVFWD), left ventricular free-wall thickness in systole (LVFWS), interventricular septal thickness in diastole (IVSD), interventricular septal thickness in systole (IVSS), shortening fraction (SF), and left atrial dimension-to-aortic diameter (LA/Ao).

| Position of dog | Operator | LVDD              | LVSD              | LVFWD                | LVFWS                | IVSD                 | IVSS                  | SF                 | LA/Ao                |
|-----------------|----------|-------------------|-------------------|----------------------|----------------------|----------------------|-----------------------|--------------------|----------------------|
| Recumbent       | 1        | 37 ± 3.1<br>(8.2) | 25 ± 2.0<br>(8.0) | 8.7 ± 1.26<br>(14.5) | 13.3 ± 1.30<br>(9.8) | 8.7 ± 1.48<br>(17.0) | 13.5 ± 1.55<br>(11.5) | 34 ± 5.1<br>(14.9) | 1.3 ± 0.35<br>(26.6) |
|                 | 2        | 37 ± 1.3<br>(3.4) | 24 ± 1.2<br>(4.9) | 8.3 ± 1.05<br>(12.6) | 12.6 ± 1.14<br>(9.0) | 8.5 ± 0.67<br>(7.9)  | 13.3 ± 1.38<br>(10.4) | 36 ± 3.1<br>(8.7)  | 1.0 ± 0.11<br>(11.0) |
| Standing        | 1        | 38 ± 1.9<br>(4.9) | 26 ± 1.8<br>(7.0) | 8.8 ± 1.10<br>(12.4) | 12.9 ± 1.26<br>(9.8) | 8.8 ± 1.90<br>(21.6) | 13.3 ± 2.10<br>(15.8) | 34 ± 4.3<br>(12.7) | 1.2 ± 0.16<br>(13.2) |
|                 | 2        | 38 ± 1.5<br>(3.9) | 25 ± 1.1<br>(4.4) | 8.3 ± 0.87<br>(10.4) | 12.5 ± 0.75<br>(6.0) | 8.7 ± 1.34<br>(15.4) | 13.1 ± 1.55<br>(11.8) | 36 ± 3.3<br>(9.2)  | 1.1 ± 0.11<br>(10.4) |

Values are expressed as mean ± SD (coefficient of variation). Operator 1 was accustomed to performing echocardiographic examinations with dogs in a recumbent position, and operator 2 was accustomed to performing examinations with dogs in a standing position.

where  $Y_{ijkl}$  is the value measured for dog  $i$  ( $i = 1$  to 4) in position  $j$  ( $j =$  standing or recumbent) by operator  $k$  ( $k = 1$  or 2) on cardiac cycle  $l$  ( $l = 1$  to 5). The dog  $\times$  position<sub>ij</sub>, dog  $\times$  operator<sub>ik</sub>, and position  $\times$  operator<sub>jk</sub> terms were the interaction terms between dog and position, dog and operator, and position and operator, respectively. Model error was described by the term  $e_{ijkl}$ . The level of significance was set at  $P < 0.05$ . The mean, SD, and coefficient of variation (CV) were determined for each echocardiographic variable according to the position of the dog and the operator.

## Results

Mean ± SD duration of the echocardiographic examinations was similar for both observers ( $5.9 \pm 2.2$  minutes and  $4.9 \pm 1.8$  minutes for observers 1 and 2, respectively) and positions ( $5.2 \pm 1.5$  minutes and  $5.6 \pm 2.4$  minutes for dogs in recumbent and standing positions, respectively). Mean ± SD and CV of the echocardiographic variables measured in the 2 positions by each operator were summarized (Table 1). Mean values were similar between operator and positions; mean differences between observers for each position of the LV diameters, SF, and LA/Ao were ≤ 1 mm, 2%, and ≤ 0.3, respectively. Values for CVs ranged from 3.4% to 26.6% but were < 15% for all but 5 values. Nevertheless, for both operators, the CVs indicated better repeatability of measurements of LVSD, LVFWD, LVFWS, and LA/Ao from dogs examined in the standing position and for IVSD and IVSS in dogs in the recumbent position. For other variables, it could not be concluded that 1 position was better than the other. Surprisingly, observer 1, who was more skilled in examining recumbent dogs, had a higher repeatability for measurement of LVDD in standing dogs, whereas the repeatability for SF measured by operator 2 was higher in recumbent dogs.

A significant effect of position on repeatability was observed for LVDD ( $P < 0.001$ ) and LVSD ( $P < 0.001$ ). A significant effect of operator was observed for LVSD ( $P < 0.001$ ), LVFWD ( $P < 0.001$ ), LVFWS ( $P < 0.001$ ), and SF ( $P < 0.01$ ). No significant association between position and operator was detected for any echocardiographic variable. In contrast, there were significant associations between position and dog for LVDD ( $P < 0.001$ ), LVSD ( $P < 0.001$ ), LVFWD ( $P < 0.05$ ), LVFWS ( $P = 0.01$ ), IVSD ( $P < 0.001$ ), and IVSS ( $P < 0.001$ ). Similarly, a significant association between dog and operator was observed for LVSD ( $P < 0.001$ ), LVFWS ( $P < 0.001$ ), IVSD ( $P = 0.01$ ), and SF ( $P < 0.05$ ).

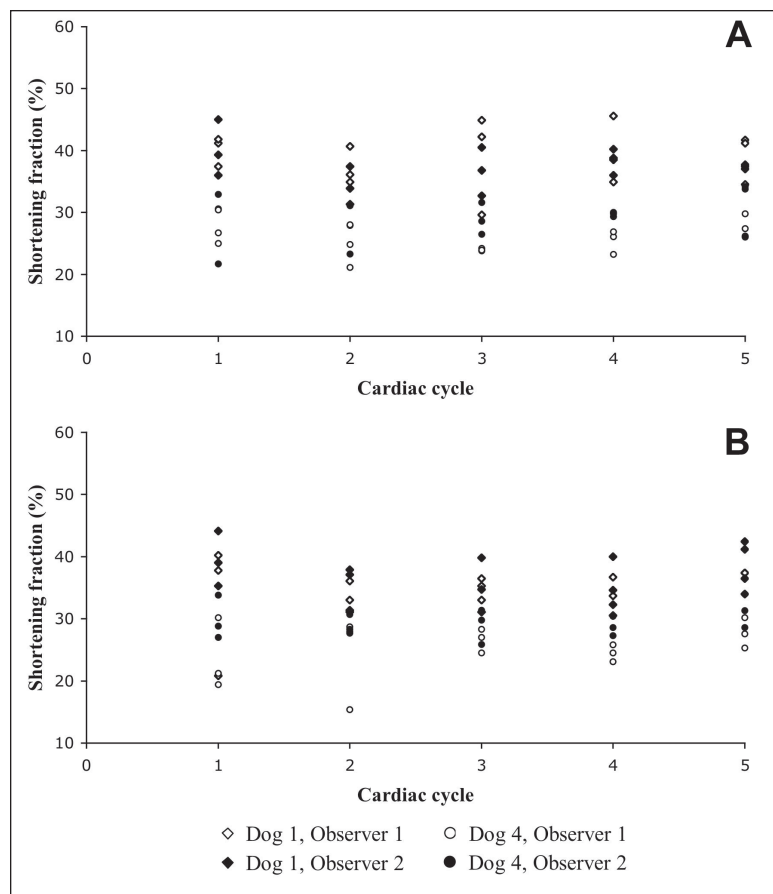


Figure 1—Values for shortening fraction determined in 5 consecutive cardiac cycles on the same day and during the same examination by operators 1 (open diamonds and circles) and 2 (solid diamonds and circles) in 2 representative dogs in the recumbent position (A) and standing position (B).

No effect of cardiac cycle was observed for any of the echocardiographic variables. Values obtained by each operator for SF on 5 different cardiac cycles in dogs in recumbent and standing positions, respectively, were summarized (Figure 1).

## Discussion

The repeatability and reproducibility of echocardiographic measurements obtained from nonsedated dogs and cats in the standing position have been reported.<sup>3,4</sup> The repeatability and reproducibility of M-mode measurements have also been analyzed in awake dogs,<sup>10</sup> anesthetized cats in lateral recumbency,<sup>11</sup> horses,<sup>12-14</sup> calves,<sup>15</sup> rats,<sup>16</sup> and humans.<sup>17-19</sup> Studies<sup>17,18</sup> in humans have assessed the number of consecutive cardiac cycles over which it is necessary to obtain images for representative values of left ventricular parameters in M-mode. However, to our knowledge, ours is the first study to assess the effects of animal position and number of repeated measurements on echocardiographic variables in dogs.

The primary aim of the study was not to document the biological causes of variation in echocardiographic variables but rather to assess the intra- and interobserver variability according to the dog's position. We hypothesized that if the same dog was examined several times, the only sources of variation would be the

method of measurement and the positions of the dog and observer; biological factors pertaining to the dog itself would remain constant throughout the period of testing. Therefore, there was no need for a large number of dogs, but a large number of measurements were obtained from the same animals by the same investigators. Healthy dogs were selected to avoid any cardiac morphologic abnormalities that might have changed the standard 2-D and M-mode echocardiographic views. Nevertheless, dogs with different cardiac sizes were used. To ensure accurate comparison of the 2 examination positions, observers were chosen for their similar high levels of experience in echocardiographic imaging but different habits of patient positioning. To exclude the influence of 1 measurement on the following one, the operators were prevented from seeing the values for measurements on the ultrasound screen. To simulate the conditions of a clinical situation or a clinical trial, we used dogs that were neither familiar with the examination procedure nor habituated to the observers and examinations were performed in the usual echocardiographic room, which was near the consulting room. Mean duration of the echocardiographic examinations and mean values of the echocardiographic variables were similar between operators and positions, confirming the comparable expertise of both observers for each position. The repeatability (within-day variability) of

each method (ie, 1 position vs the other) was determined for each operator. Poor repeatability for a given variable would have made that position impracticable, but all CV values were < 15% except for IVSD (7.9% to 21.6%), IVSS (10.4% to 15.8%), and LA/Ao (10.4% to 26.6%).

One of the main objectives of our study was to evaluate the effect of dog positioning on the within-day intraobserver variability of echocardiographic measurements. Repeatability was good for both positions and both observers, as indicated by CV values < 15% for 27 of 32 variables (8 variables for 2 observers and 2 positions). The lowest CV values were observed for LV diameters with observer 2 (LVDD and LVSD: 3.9% and 4.4% in standing dogs, 3.4% and 4.9% in recumbent dogs, respectively).

For both operators, repeatability was better for 4 of 8 variables (LVSD, LVFWD, LVFWS, and LA/Ao) when the measurements were made in standing dogs, whereas the recumbent position was better for 2 variables (IVSD and IVSS). Results indicate that although most veterinary cardiologists prefer dogs to be positioned in lateral recumbency, use of the standing position yields similar results and provides similar or better within-day CVs for all variables except interventricular wall thickness. These data agree with results of previous echocardiographic studies<sup>3,4</sup> performed in awake standing dogs and cats.

A significant effect of position was detected for 2 variables (LVDD and LVSD), with lower values being measured in recumbent dogs. Nevertheless, this difference was evident because the CV values were lowest for these 2 variables. The difference, although significant, was so small (mean difference, 1 mm for each observer) that it could not affect the interpretation of echocardiographic results in practice and was consequently of limited clinical relevance.

A significant operator effect was observed for 4 measurements (LVSD, LVFWD, LVFWS, and SF). This could be explained by a difference in observer familiarity with one of the positions because an operator effect has already been described in canine echocardiography among operators with different levels of experience.<sup>4</sup> However, for most of these variables, the CV values were small (ie, < 15%).

No significant interaction was detected between position and operator, indicating that either of the observers might over- or underestimate a given variable, depending on the position of the dog. Moreover, we found that 2 weeks of training was sufficient for an experienced observer accustomed to obtaining measurements in dogs in 1 position to obtain good within-day variability with dogs in the other position.

A significant interaction between dog and operator was observed for certain echocardiographic variables (LVSD, LVFWS, IVSD, and SF). A similar interaction has also been reported for echocardiographic imaging in cats<sup>3</sup> and dogs.<sup>4</sup> This interaction indicates that 1 observer might over- or underestimate a given variable, compared with data obtained by the other observer, depending on the dog under evaluation. Therefore, in any follow-up echocardiographic examination, a dog should be examined by the same observer for correct interpretation of variation in echocardiographic values.

A significant interaction between position and dog was also observed for 6 of 8 variables (ie, all but LA/Ao and SF). This finding suggests that such variables might be over- or underestimated in a given dog according to its position during the echocardiographic evaluation and implies that dogs should always be examined in the same position during follow-up examinations.

Echocardiographic measurements calculated as a mean of measurements from 3 to 5 consecutive cardiac cycles are commonly used in dogs with sinus rhythm.<sup>1</sup> However, no cardiac cycle effect was evident in the present study, which indicated that between-cycle variability cannot be dissociated from all of the other uncontrolled sources of variation (eg, noise). Because the variance of the mean of *n* measurements is less than the variance of the individual values obtained (ie, variance of the mean of the measurements is actually equal to the variance of individual cardiac cycles divided by *n*), it is therefore less than the uncontrolled variability. This finding indicates that, under the conditions of our study, no significant improvement is obtained by calculating the mean of multiple measurements if the observer is suitably trained. However, use of the mean of multiple measurements may improve interpretation of echocardiographic variables under certain conditions (eg, less experienced observers or cardiac disease conditions), but such conditions must

be investigated and defined. One of the limits of our study was that only healthy dogs were assessed. The SF may vary considerably with arrhythmias (eg, atrial fibrillation). However, to our knowledge, there are no published data comparing the extent of beat-to-beat variation with the within-day variability of conventional echocardiographic variables in dogs with arrhythmias. Further investigation in dogs with disease is necessary to determine the optimal number of cardiac cycles over which measurements are required before making recommendations on this point.

Whether the variability of the echocardiographic examination is clinically relevant or not is an important issue. Because a given variation is significant does not necessarily mean that it is clinically relevant. For example, if the CV of the measurement for LVDD is 5% but the clinician considers that a clinically relevant variation is at least 20%, the variability in the measurement is not clinically relevant. Unfortunately, such cutoff values for relevant variation in echocardiographic variables in dogs have not been published.

These results could affect the designing of drug clinical trials.<sup>4</sup> Quantitative clinical variables such as echocardiographic variables are used as end points in clinical trials assessing the effects of certain drugs (including enalapril<sup>20,21</sup> and hydralazine<sup>20</sup>) on heart function in dogs. In a previous study,<sup>4</sup> the within- and between-day intraobserver variability in echocardiographic measurements considerably influenced the minimum number of animals required to detect a significant difference in an echocardiographic variable between 2 groups of animals (eg, treated vs placebo)—the lower the CV value, the lower the number of animals needed. For example, if the within-observer between-examination CV value for SF is 10% and 21% for 2 observers, the number of animals per group (treated or placebo) required to detect an absolute difference of 4% in SF associated with a new treatment would be 46 for a diplomate of the European College of Veterinary Internal Medicine (cardiology) and 96 for a general practitioner. The data from that study and the present investigation indicate that the echocardiographic examination procedure, like any other quantitative technique, should be validated and standardized, including the calculation of values for CV and SD and the number and expertise of observers. Moreover, the same observer and dog position should be consistently used during follow-up examinations.

Results of this study are only valid under the conditions described with respect to the ultrasound machines used, the observers, and the dogs and may differ in other circumstances. Because only healthy dogs were used in the present study, our data may not apply to dogs with heart disease. Different results might be obtained with other breeds of dogs. A given position for examination might be more suitable for a particular breed because of breed-specific morphologic characteristics of the thorax. For example, it is possible that in dogs with a large thorax, such as English Bulldogs, higher-quality images might be obtained with the dog in lateral recumbency rather than the standing position.

Transthoracic 2-D and M-mode echocardiography can be performed on dogs in the standing position, and



the repeatability of measurements is similar to or better than those obtained in recumbent dogs. Operators who examine dogs in lateral recumbency should gain experience in standing dogs because the latter position is useful and may be preferable in certain clinical situations (eg, stressed or dyspneic patients). Our data indicate that single measurements of each variable are sufficient when the observer is suitably trained, but further investigations are needed.

- a. Vingmed Vivid 5, General Electric Medical System, Waukesha, Wis.
- b. Systat, version 10.0, SPSS Inc, Chicago, Ill.

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