Effect of cardiac and respiratory cycles on vertebral heart score measured on fluoroscopic images of healthy dogs

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Objective—To assess the variability in vertebral heart score (VHS) measurement induced by cardiac and respiratory cycles in dogs.

Design—Prospective observational study.

Animals—14 healthy Beagles.

Procedures—Dogs underwent fluoroscopic examination by 4 observers, and VHS was measured at end-tidal inspiration and end-tidal expiration during end systole and end diastole in left and right lateral recumbency. Mean VHS was compared within and among cardiac and respiratory phases and recumbency type, and correlation between VHS and heart rate was investigated. Interobserver variability was assessed.

Results—Mean VHS for each combination of respiratory and cardiac cycle was larger on images obtained in right lateral versus left lateral recumbency. The greatest differences were observed between VHS measured in the diastolic inspiratory phase (mean ± SD, 10.59 ± 0.49 vertebral units [VU] and 10.35 ± 0.50 VU for right and left lateral recumbency, respectively) and the systolic expiratory phase (10.11 ± 0.37 VU and 9.92 ± 0.50 VU for right and left lateral recumbency, respectively). The combination of respiratory and cardiac cycles induced a maximal difference in VHS of up to 0.97 VU and 1.11 VU in the inspiratory and expiratory phases, respectively. Heart rate was not correlated with the difference between VHS in systolic and diastolic phases.

Conclusions and Clinical Relevance—Clinicians should be aware of the potential influence of these factors when assessing VHS in dogs; in addition to allowing optimal pulmonary assessment, consistently taking radiographs at end-inspiratory tidal volume may help to limit VHS variability attributable to the respiratory cycle. Further research is needed to assess the effects of cardiac and respiratory phases on VHS in dogs with cardiac or respiratory disease. (J Am Vet Med Assoc 2015;246:1091–1097)
cardiac cycles on VHS determined by means of thoracic fluoroscopy in healthy dogs. We hypothesized that VHS would be greater in diastole than in systole and that the effect of respiration at rest would be minimal. We also hypothesized that a slower heart rate would induce a greater difference in VHS between systolic and diastolic phases because of increased preload.

Materials and Methods

Animals—Fourteen healthy adult Beagles were included in the study between January 1, 2012, and January 31, 2013. Median weight of these dogs was 10 kg (22 lb; range, 6.2 to 11.6 kg [13.6 to 25.9 lb]). Age and sex were not recorded for all dogs. Dogs were from the teaching colony of the Faculty of Veterinary Medicine of the University of Montreal. The institutional ethics committee approved the study protocol in accordance with the Canadian Council of Animal Welfare. The dogs had no history of cardiac or pulmonary disease. For each dog, a complete clinical examination with cardiac auscultation was performed. If no abnormality was detected, ECG, echocardiography, and fluoroscopic assessment were performed. Dogs were included in the study if they had no detectable heart murmur and no abnormality identified on cardiac evaluation.

Echocardiography and ECG—The echocardiographic examination (2-D, M-mode, and color flow Doppler) was performed on all dogs without sedation. An ultrasound unit equipped with a 1.5- to 4.0-MHz or 3.5- to 8.0-MHz phased-array transducer and simultaneous ECG display was used. During echocardiography, animals were positioned in lateral recumbency while standard views (right parasternal, left apical, and subcostal) were obtained. One operator (RJ) performed all echocardiographic imaging. Dogs underwent ECG for ≥30 seconds immediately prior to fluoroscopic recordings to evaluate for cardiac arrhythmia and to obtain heart rate at the time of the procedure. This allowed evaluation of the effect of heart rate on VHS measurements. Dogs were not sedated and were physically restrained with sandbags or additional manual restraint when needed.

Fluoroscopy—Thoracic fluoroscopic loops were obtained for 10 seconds, first in right lateral and then in left lateral recumbency. Imaging parameters were a round field of view, 512 × 512 matrix with rectangular shutters, pulsed radiation mode at a frame rate of 15 images/s, and mean kVp of 53 to 62. Images were sent to a commercial picture archiving and communication system and later retrieved with DICOM (Digital Imaging and Communications in Medicine) software for analysis.

Image analysis—Vertebral heart scores were determined as previously described. Briefly, the long axis of the cardiac silhouette was measured with a digital caliper from the ventral border of the carina to the cardiac apex. The maximal cardiac short axis was measured perpendicularly to the long axis, starting just ventral to the caval vena cava. Both measurements were scaled to a number of thoracic vertebrae, starting at the cranial aspect of the T4 vertebral body. Measurements were made for each type of recumbency at the time of the smallest and largest subjectively visualized cardiac silhouette, considered to represent end-systolic and end-diastolic stages, respectively. These measurements were performed at resting end-tidal inspiration and end-tidal expiration (8 measurements/dog [4 each for right and left lateral recumbency]). One investigator selected images to be examined by all observers. Vertebral heart score was determined for each image individually by 4 observers with different levels of experience: a board-certified radiologist, third-year and second-year radiology residents, and an intern. Each observer was blinded to the findings of other observers at the time of assessment.

Statistical analysis—The Anderson-Darling test revealed no significant deviation from normality in VHS values; thus, mean and SD values are provided. Mean values from all 4 observers were calculated for each dog for each combination of recumbency type, cardiac phase, and respiratory phase. Within and between cardiac and respiratory cycle phases, mean VHS values for right versus left lateral recumbency, systolic versus diastolic phases, and inspiration versus expiration phases as well as maximal mean VHS differences were calculated and compared by means of paired Wilcoxon signed rank tests. The greatest mean change in VHS induced by the combination of respiratory and cardiac phases was calculated from the means of all diastolic-systolic VHS differences for each dog (in both recumbencies pooled together, with values from all observers) as (mean difference in VHS) + 2 SD. Intraclass correlation coefficient for quantitative VHS measurement was assessed with a mixed linear model to evaluate interobserver agreement across all observers. The same model was used to evaluate the agreement between each observer and the most experienced observer (KA). Interobserver agreement was also assessed by means of modified Bland-Altman analysis for each pair of observers to determine the mean difference and potential systematic bias between observers. The modified method takes repeated measures (8 for each dog) into account when calculating the limits of agreement. Mean interobserver VHS variability was also calculated by pairs and overall. A Pearson correlation coefficient was calculated to examine the relationship between heart rate and the VHS difference between systolic and diastolic phases. A statistical software package was used for all analyses. Values of P ≤ 0.05 were considered significant.

Results

VHS measurements—Mean ± SD VHS were summarized (Table 1). Because 2 observers were unable to locate the cranial border of T4 in some images, the number of VHS measurements was 400 of a potential 448. Maximal VHS ranges for each dog (including all cardiac and respiratory phase combinations) measured by the most experienced observer (KA) were plotted (Figure 1). Diastolic VHS were significantly (P < 0.05) greater than systolic VHS at the same respiratory cycle stage in both left and right lateral recumbency. All combinations of cardiac and respiratory phase VHS measurements were larger on right lateral images, com-
pared with left lateral images. Representative images were obtained for each cardiac and respiratory phase combination (Figure 2).

Evaluation of raw data from all observers revealed that 33 of 400 (8.3%) VHS were ≥ 11.0 VU. Nine of 14 dogs had at least 1 VHS ≥ 11 VU, and 8 of these 9 dogs had from 2 to 6 VHS ≥ 11 VU. One dog had 10 VHS measurements ≥ 11 VU. Regarding the respiratory cycle, 18 of 33 (55%) VHS ≥ 11 VU were measured at end-tidal inspiration and 15 (45%) were measured at end-tidal expiration. Most of the VHS ≥ 11 VU were obtained at end diastole (25/33 [76%]). There were 17 of 33 (52%) VHS ≥ 11 VU measured in right lateral images and 16 (48%) measured in left lateral images. Finally, most of the VHS ≥ 11 VU (25/33 [76%]) were obtained by 2 operators.

Effects of cardiac and respiratory cycles on VHS—
Inspiratory systolic VHS was significantly greater than expiratory systolic VHS (Table 1). Conversely, inspiratory diastolic VHS was not significantly different from expiratory diastolic VHS.

Differences between mean diastolic and systolic VHS within each respiratory phase and differences between inspiratory and expiratory VHS within each cardiac phase were not significantly different for right versus left lateral images (Table 2). When both recumbencies were considered together, mean ± SD difference between systolic and diastolic VHS was significantly higher for expiratory (0.43 ± 0.34 VU) than for inspiratory (0.29 ± 0.34 VU) images. In inspiratory images, the greatest mean difference in VHS between systolic and diastolic phases among individual dogs was calculated as 0.97 VU; in expiratory images, this value was calculated as 1.11 VU. The overall systolic to diastolic VHS mean difference was 0.36 ± 0.42 VU, and overall inspiratory to expiratory VHS mean difference was 0.10 ± 0.31 VU.

The mean difference in VHS between the systolic and diastolic phases was significantly lower for inspiratory versus expiratory images from dogs in left, but not right, lateral recumbency (Table 2). The mean dif-

Table 1—Mean ± SD VHS (VU) in all cardiac and respiratory phases determined individually by 4 observers with different levels of experience: a board-certified radiologist, third-year and second-year radiology residents, and an intern.

<table>
<thead>
<tr>
<th>Respiratory and cardiac phase</th>
<th>Recumbency type</th>
<th>Right lateral</th>
<th>Left lateral</th>
</tr>
</thead>
<tbody>
<tr>
<td>End-tidal inspiration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End systole</td>
<td></td>
<td>10.29 ± 0.35a</td>
<td>10.08 ± 0.42a</td>
</tr>
<tr>
<td>End diastole</td>
<td></td>
<td>10.11 ± 0.37b</td>
<td>9.92 ± 0.50b</td>
</tr>
<tr>
<td>End-tidal expiration</td>
<td></td>
<td>10.11 ± 0.37b</td>
<td>9.92 ± 0.50b</td>
</tr>
<tr>
<td>End systole</td>
<td></td>
<td>10.53 ± 0.40c</td>
<td>10.35 ± 0.46c</td>
</tr>
<tr>
<td>End diastole</td>
<td></td>
<td>10.59 ± 0.49a</td>
<td>10.35 ± 0.50a</td>
</tr>
</tbody>
</table>

Scores were determined by evaluation of fluoroscopic images. Images with the smallest and greatest subjectively visualized cardiac silhouettes were considered to represent end-systolic and end-diastolic stages, respectively.

Within a column, all possible pairs of comparisons were made and values with the same superscript letter are not significantly (P > 0.05) different (14 mean values [1 per dog] for each group, compared by means of paired Wilcoxon signed rank tests). Within a row, values for right lateral and left lateral recumbency are all significantly (P ≤ 0.05) different.
Figure 2—Representative fluoroscopic images of the thorax of a healthy Beagle in right lateral recumbency (A–D). The VHS at 4 combinations of cardiac and respiratory stages (end diastole at end-tidal expiration [A], end systole at end-tidal expiration [B], end diastole at end-tidal inspiration [C], and end systole at end-tidal inspiration [D]) are shown.
difference in VHS between the inspiratory and expiratory phases was also significantly greater for systolic than for diastolic images of dogs in left, but not right, lateral recumbency.

Association between heart rate and difference in VHS between cardiac phases—Mean and SD heart rate at the start of fluoroscopic examination was 103 ± 19 beats/min (range, 78 to 142 beats/min). Mean heart rate was not correlated with the difference in VHS between the diastolic and systolic phase for any observer.

Interobserver measurement agreement—The overall intraclass correlation coefficient for interobserver variability was moderate (0.45). Relative to variability of the most experienced observer (KA), all intraclass correlation coefficients were similar for each other observer (range, 0.36 to 0.40). According to the Bland-Altman analysis, there was a significant systematic bias for 6 of 6 possible combinations of observers. The smallest significant bias was 0.11 VU and the greatest was 0.44 VU, without any apparent relationship with the level of experience. The mean VHS variability among all observers was 0.42 ± 0.34 VU, ranging from 0.36 ± 0.33 VU and 0.53 ± 0.40 VU for different pairs of observers.

Discussion

Although the timing of a thoracic radiograph can often be controlled in regard to the respiratory cycle, timing cannot be controlled in regard to the cardiac cycle. To the authors’ knowledge, this is the first study to evaluate the influence of the cardiac cycle on VHS in dogs. Overall, the cardiac cycle impacted the VHS, with the mean ± SD difference between end-systolic and end-diastolic measurements being 0.29 ± 0.34 VU at end-tidal inspiration. Taking into account the SD, this implies that a VHS difference of up to almost 1 VU on 2 sets of radiographs for the same dog might be attributable to the effect of cardiac cycle. Moreover, the mean ± SD difference between systolic and diastolic VHS was significantly higher for expiratory (0.43 ± 0.34 VU) versus inspiratory (0.29 ± 0.34 VU) images, enhancing the importance of taking thoracic radiographs at end-tidal inspiration. Taking into account the suggested threshold value for healthy Beagles, 9 33 of 400 (8.3%) VHS measurements overall (from all observers and all combinations of cardiac and respiratory phases assessed) were ≥ 11.0 VU, mostly in diastole as expected from other results of the study, with variable apparent impact of individual observers and dogs. This underlines a potential risk of erroneously considering cardiomegaly in a small number of healthy patients.

In all phases of the cardiac and respiratory cycles, the VHS was mildly but significantly greater in right than in left lateral recumbency, by approximately 0.2 VU, as reported previously. Indeed, there was no difference between the left and right lateral projections in a few studies,1–3 but in others, the score was mildly but significantly higher on the right lateral projection versus the left lateral projection in Beagles (10.5 ± 0.4 VU vs 10.2 ± 0.4 VU, respectively),9 Whippets (11.3 ± 0.3 VU vs 11.0 ± 0.5 VU, respectively),8 and a population of various breeds (9.8 ± 0.6 VU vs 9.5 ± 0.8 VU, respectively).8–11 It is well-known that the cardiac silhouette has a different shape on a right lateral versus a left lateral radiographic projection. The effect of magnification may also be greater on images obtained in right lateral recumbency because of the leftward intrathoracic position of the heart. The slight differences in VHS may reflect this.

Interestingly, systolic VHS was significantly higher in inspiratory images than in expiratory images. This difference can be explained by some physiologic concepts.19,20 Briefly, increasing the depth of respiration promotes venous return and therefore enhances cardiac output.19 Respiratory activity affects venous return through changes in right atrial pressure.21 Increasing right atrial pressure impedes venous return and vice versa. Respiratory activity can also affect venous return either directly by vena cava compression or indirectly by changing cardiac preload. Pressures in the right atrium and thoracic vena cava are very dependent on intrapleural pressure.20 During inspiration, the thoracic wall expands and the diaphragm moves caudally. This causes intrapleural pressure to become more negative, which leads to expansion of the lungs, cardiac chambers, and thoracic cranial and caudal vena cava. This expansion causes intravascular and right atrial pressure to fall, leading to cardiac chamber expansion and an increase in cardiac preload and stroke volume through the Frank-Starling mechanism.19–21 During expiration, the opposite occurs.

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**Table 2—Mean ± SD differences in VHS (VU) determined by all 4 observers for dogs in left and right lateral recumbency.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Right lateral</th>
<th>Left lateral</th>
</tr>
</thead>
<tbody>
<tr>
<td>End-diastolic – end-systolic measurement</td>
<td>0.30 ± 0.43</td>
<td>0.27 ± 0.42</td>
</tr>
<tr>
<td>End-tidal inspiration</td>
<td>0.37 ± 0.40</td>
<td>0.43 ± 0.46</td>
</tr>
<tr>
<td>End-tidal expiration</td>
<td>0.32 ± 0.39</td>
<td>0.32 ± 0.43</td>
</tr>
<tr>
<td>End-systole</td>
<td>0.18 ± 0.35</td>
<td>0.17 ± 0.29</td>
</tr>
<tr>
<td>End diastole</td>
<td>0.06 ± 0.32</td>
<td>0.00 ± 0.28</td>
</tr>
<tr>
<td>Greatest difference</td>
<td>0.48 ± 0.39</td>
<td>0.44 ± 0.36</td>
</tr>
</tbody>
</table>

*Within a column, all possible pairs of comparisons were made and values with the same superscript letter are not significantly (P ≥ 0.05) different (14 mean values [1 per dog] for each group, compared by means of paired Wilcoxon signed rank tests). There was no significant difference between VHS measured in left versus right lateral images for any of the comparisons.*
The left side of the heart responds differently to the respiratory cycle than does the right side. During inspiration, expansion of the lungs and pulmonary tissues causes pulmonary blood volume to increase, which transiently decreases blood flow from the lungs to the left atrium. Thus, left ventricular filling actually decreases during inspiration. In contrast, during expiration, lung deflation causes blood flow to increase from the lungs to the left atrium, which increases left ventricular filling. The net effect of increased rate and depth of respiration is an increase in left ventricular stroke volume and cardiac output. Nontypical respiratory activity such as application of positive-pressure ventilation impedes and therefore reduces venous return and cardiac output. This explains why, in anesthetized dogs, increased manual inflation decreased VHS by a mean of 0.24 VU.21

The difference between VHS in systolic and diastolic phases was significantly greater in expiratory versus inspiratory images from dogs in left lateral recumbency. Some physiologic concepts may help to explain these findings. A slightly negative pressure is present at the beginning of inspiration (approx. −5 cm H₂O, which is the amount of negative intrathoracic negative pressure required to hold the lungs open).20 The previously described expansion of the chest wall during normal inspiration creates a more negative pressure (approx −7.5 cm H₂O),21 causing alteration in cardiac chamber geometry21 as well as transient inspiratory pooling of blood in the lungs (suction effect).21 Results of a recent MRI study23 revealed that inspiration results in a transient increase in right ventricular volume and a reciprocal decrease in left ventricular volume. It can be hypothesized that similar changes are present in clinically normal dogs and that alteration in cardiac chamber geometry combined with pooling of blood in the lungs during inspiration can have some repercussions on cardiac chamber size and filling, accounting for a smaller difference between VHS in systolic and diastolic phases during inspiration.

There was no apparent effect of heart rate on the difference in VHS between the systolic and diastolic phase, probably because the range of heart rates at rest was too limited to detect any underlying change in the cardiac preload relative to individual variability. Interestingly, the calculated mean difference in VHS for systolic versus diastolic phases (0.36 VU) was similar to the greatest interobserver systematic bias for VHS measurements (0.44 VU). Despite a lower mean interobserver variability in the present study (0.42 ± 0.34), compared with that in a previous study2 (1.05 ± 0.32 VU), VHS remains dependent on individual observer selection of reference points and transformation into VU.22 Interobserver variability is typically lower than interobserver variability and was estimated between 0.6 and 0.8 VU in another study.13 This suggests that longitudinal studies should be conducted by the same observer, especially given the relatively mild expected increase in VHS (0.17 VU/mo) reported for at least 1 breed of dog during the last months before onset of congestive heart failure.12

The present study had some limitations. An increased rate of error in VHS measurements may have existed because of the lower resolution and contrast of fluoroscopic images, compared with digital radiographs. The cranial aspect of T4 was indeed occasionally difficult to discern in some dogs. The applicability of our results may be limited to Beagles, and differences in VHS determined in systolic and diastolic images may be dependent on breed size and chest conformation. Although similar changes might be anticipated with other breeds, the magnitude might be different and this would require further investigations. Furthermore, whether similar phasic changes occur in dogs with eccentric hypertrophy remains unknown. Volume overload might actually limit the amplitude of maximal VHS difference. Therefore, our results can only apply to healthy dogs and a similar study should be conducted in a cohort of dogs with cardiac disease.

The findings of this study emphasized that the cardiac cycle and, to a lesser extent, respiratory cycle have moderate yet non-negligible effects on VHS determination. The influence of these cycles may potentially explain small variations in VHS measurement when monitoring cardiac patients; however, this was not specifically evaluated and further research is needed. To minimize variabiliy, the same operator should make comparative assessments and recumbency should be consistent in the images used.

References

11. Nakayama H, Nakayama T, Haminsya RL. Correlation of cardiac enlargement as assessed by vertebral heart size and echocardiographic and electrocardiographic findings in dogs with...

From this month’s AJVR

Stability of hemostatic proteins in canine fresh-frozen plasma thawed with a modified commercial microwave warmer or warm water bath

Medora B. Pashmakova et al

Objective—To compare stability of hemostatic proteins in canine fresh-frozen plasma (FFP) thawed with a modified commercial microwave warmer (MCM) or warm water bath (37°C; WWB) or at room temperature (22°C).

Sample—Fresh-frozen plasma obtained from 8 canine donors of a commercial blood bank.

Procedures—A commercial microwave warmer was modified with a thermocouple to measure surface temperature of bags containing plasma. The MCM and a WWB were each used to concurrently thaw a 60-mL bag of plasma obtained from the same donor. Two 3-mL control aliquots of FFP from each donor were thawed to room temperature without use of a heating device. Concentrations of hemostatic proteins, albumin, and D-dimers; prothrombin time (PT); and activated partial thromboplastin time (aPTT) were determined for all samples.

Results—Significant decreases in concentrations of factors II, IX, X, XI, fibrinogen, von Willebrand factor, antithrombin, protein C, and albumin and significant increases in PT and aPTT were detected for plasma thawed with the MCM, compared with results for samples thawed with the WWB. Concentrations of factors VII, VIII, and XII were not significantly different between plasma thawed with the MCM and WWB. Concentrations of D-dimers were above the reference range for all thawed samples regardless of thawing method. No significant differences in factor concentrations were detected between control and WWB-thawed samples.

Conclusions and Clinical Relevance—Significant differences in hemostatic protein concentrations and coagulation times were detected for plasma thawed with an MCM but not between control and WWB-thawed samples. Clinical importance of these changes should be investigated. (Am J Vet Res 2015;76:420–425)