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Breed-Specific Vertebral Heart Scale for the Dachshund

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ABSTRACT

The objectives of this study were to determine a breed-specific vertebral heart scale (VHS) range for the dachshund and compare results to the established reference range of 9.7 ± 0.5, calculate inter-observer variability, and correlate VHS with echocardiography. Fifty-one normal dachshunds had radiographs and an echocardiogram performed. Five observers measured VHS to the nearest 0.25 vertebra. The data was analyzed using one-way analysis of variance, Wilcoxon Rank Sum test, Mann-Whitney rank sum test, calculation of reference and confidence intervals, Spearman rank-order correlations, and generation of intra-class correlations and confidence intervals. $P < .05$ was considered significant. The median for right lateral VHS was significantly larger than left (10.3 [range 9.25–11.55] versus 10.1 [range, 8.7–11.31], $p < .0001$). VHS for females was significantly larger than for males (left: 10.56 [9.2–11.31] versus 9.74 [8.7–10.88] and right: 10.8 [9.5–11.55] versus 9.99 [9.25–10.8], $p = .0002$). Observer consistency was high with an intra-class correlation coefficient of 0.95. No significant correlation was found between left atrial echocardiographic parameters and VHS. Results indicate normal dachshunds have a median VHS above the published generic canine reference range, and VHS can be reliably performed by observers with varying degrees of clinical experience. (*J Am Anim Hosp Assoc* 2017; 53:73–79. DOI 10.5326/JAAHA-MS-6474)

Introduction

Thoracic radiography is the most common method used for evaluation of heart size in veterinary medicine. Traditionally, it has been subjectively evaluated using estimations based on anatomic ratios such as: (1) the width of the cardiac silhouette should be approximately 3–3.5 intercostal spaces on the lateral view; (2) the height of the silhouette should not exceed 60% of the thoracic height on lateral views; and (3) the maximal width of the silhouette

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LA (left atrium); LA/Ao (left atrium to aortic valve diameter ratio); MMVD (myxomatous mitral valve disease); SD (standard deviation); 2D (two-dimensional); VHS (vertebral heart scale)

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should not exceed the width of the hemithorax at the level of the 9th rib on the dorsoventral/ventrodorsal view.[1] However, inexperienced observers may have difficulty identifying cardiomegaly using only subjective guidelines, especially as there is significant diversity in thoracic conformation between breeds.[2–4] The vertebral heart scale (VHS) is an objective method to evaluate the size of the cardiac silhouette and quantitate progressive changes over time. Vertebral heart scale compares the summed long and short axes dimensions of the cardiac silhouette to the vertebral bodies.[3–12] The method was originally developed using 100 clinically normal dogs of various breeds. The mean VHS on lateral radiographs was 9.7 ± 0.5 with a range of 8.5–10.6. In 98% of the dogs, the VHS was ≤10.5, which was suggested to be the normal upper limit for heart size.[4]

The VHS has proven to be a reliable and repeatable method to calculate the size of the cardiac silhouette with minimal inter-observer variability.[9] However, VHS measurements are affected by the diverse cardiac conformations between canine breeds. The original reference range, determined by Buchanan and Bucheler, has been shown to be inaccurate for several breeds whose normal VHS ranges exceed those originally described. These breeds include the beagle, greyhound, whippet, boxer, Labrador retriever, Cavalier King Charles spaniel, pug, Pomeranian, bulldog, and Boston terrier.[3,6,8,12,13] In a recent retrospective study, the dachshund was found to have a mean VHS of 9.7.[13] In this study, dogs with subjectively enlarged cardiac silhouettes noted in the radiographic reports were excluded from analysis, likely selecting for dogs with a smaller VHS. Additionally, the effects of anesthesia and non-cardiac diseases which could contribute to volume under-loading were not considered in this study. Given the normal variation in canine heart size and thoracic conformation, development of breed-specific VHS values, particularly in breeds predisposed to cardiac disease, will improve accurate radiographic interpretation of the cardiac silhouette.

Myxomatous mitral valve disease (MMVD) is the most common heart disease in dogs and the dachshund is a breed known to be particularly predisposed.[14–16] Morbidity encountered with the disease includes exercise intolerance, dyspnea, syncope, pulmonary hypertension, and atrial fibrillation. Although affected animals may remain asymptomatic for years; nonetheless, congestive heart failure is a common sequela.[17] Further, the rate of MMVD progression is variable between individuals and should be monitored regularly with thoracic radiographs to assess for increasing cardiomegaly and potential ensuing heart failure.

The aims of this study were to: (1) establish a breed-specific VHS range for the normal dachshund and determine if it falls within the original canine reference range of 9.7 ± 0.5; (2) assess inter-observer variability between observers of varying levels of clinical training; and (3) correlate the radiographic VHS with echocardiographic measures of the left atrium (LA) dimension.

We hypothesized that: (1) normal dachshunds would have a mean VHS above the original reference; (2) there would be minimal inter-observer variability; and (3) VHS would be directly correlated with echocardiographic measurements of LA size.

Materials and Methods

Animals

Fifty-one privately owned, healthy dachshunds were prospectively recruited to have physical examinations, thoracic radiographs, and echocardiograms performed. Animals were recruited from faculty, staff, and students of the University of Missouri between March 2011 and March 2013. No sedation was used for any portion of this study. Dogs were considered normal if no physical examination abnormalities were identified and they were not receiving any medications which would alter circulating blood volume. This study was performed with approval of the University of Missouri animal care and use committee. Informed owner consent was obtained prior to participation in the study.

Thoracic Radiography

Vertebral heart scale measurements were performed by five independent observers of varying levels of clinical training (one each: senior veterinary student, cardiology intern, third year cardiology resident, boarded cardiologist, and boarded radiologist) for all radiographic studies on left and right lateral thoracic radiographs using post-processing digital software[a]. Observers were blinded to the echocardiographic results. Measurements were performed using the method described by Buchanan, with clarification to which landmarks were to be used.[4,9] Briefly, the long axis of the heart was measured from the ventral aspect of the carina to the most ventral aspect of the apex. The short axis was measured perpendicular to the long axis measurement, at the widest aspect of the heart. The caudal landmark for the short axis was defined as the midpoint between the dorsal and ventral borders of the caudal vena cava. The long and short axis measurements were then repositioned over the thoracic vertebrae starting at the cranial end-plate of the fourth thoracic vertebra, and measurements were recorded to the nearest 0.25 vertebra. One vertebral unit was defined as the distance from one cranial end-plate to the beginning of the following vertebral cranial end-plate (**Figure 1**).

Echocardiography

All echocardiographic examinations were performed by a single board-certified cardiologist (DMF) or by a cardiology resident

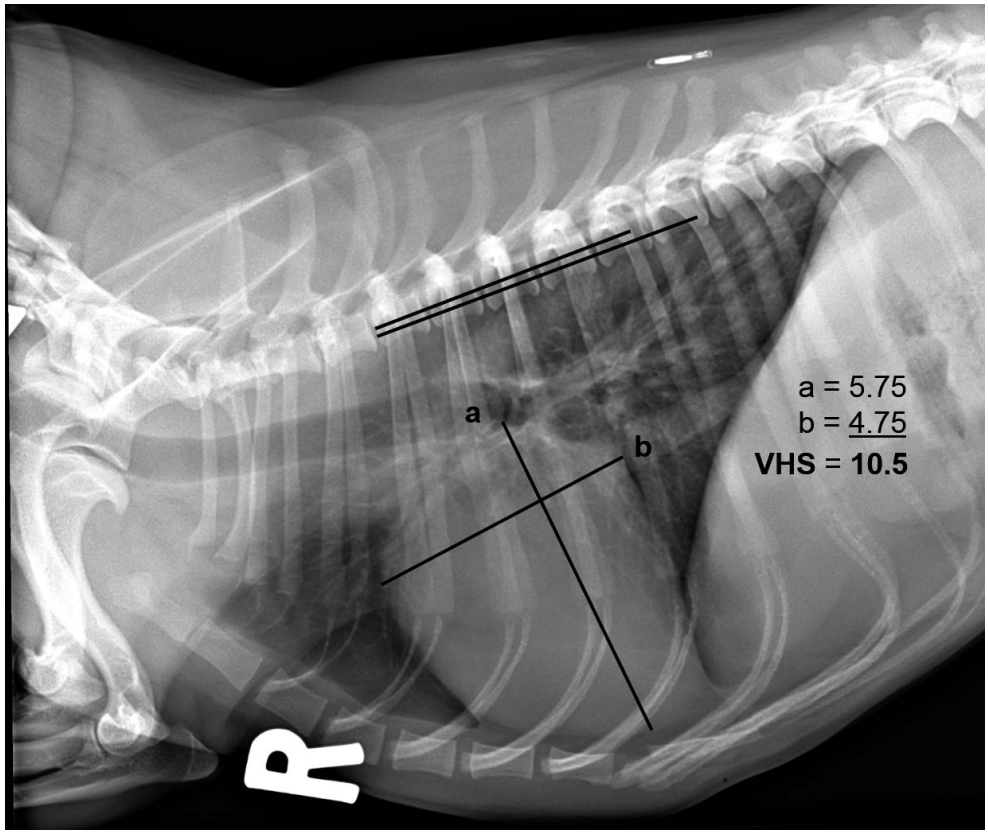

FIGURE 1 *Right lateral digital radiographic image of a normal dachshund, illustrating reference points for vertebral heart scale measurement. The cardiac long axis is measured from the ventral aspect of the carina to the apex* (a)*. The short axis is measured perpendicular to the long axis, at the widest aspect of the heart* (b)*. The caudal landmark for the short axis is the midpoint between the dorsal and ventral borders of the caudal vena cava. The long and short axis measurements are then repositioned over the thoracic vertebrae starting at the cranial end-plate of the 4th thoracic vertebra. One vertebral unit was defined as the distance from one cranial end-plate to the beginning of the following vertebral cranial end-plate.*

under the direct supervision of a board-certified cardiologist. The echocardiographer was blinded to the radiographic results. Two-dimensional (2D) and M-mode echocardiographic examinations were performed utilizing standard views in unsedated dogs.[18,19] Specific measurements of interest included: 2D LA long axis diameter using the right parasternal long axis view, M-mode LA diameter, and M-mode LA to aortic valve diameter ratio (LA/Ao) using the right parasternal short axis view at the level of the heart base. Three to five consecutive measurements were averaged for each variable.

Statistical Analyses

Initial descriptive statistics included means and standard deviations (SD) for normally distributed data and median and range for data that were not normally distributed. One-way analysis of variance was used to evaluate for differences in weight and age related to gender. Five independent observers measured VHS for each of the 51 subjects, resulting in an initial dataset consisting of n = 255 elements for each radiographic projection. These data were reviewed for entry errors and corrected as appropriate. Once outliers were evaluated and a final determination regarding the distribution of the data was made, each subject's mean score (left and right lateral projection) was calculated from the multiple assessments, resulting in reference interval datasets consisting of n = 51 values for both left and right VHS parameters.

An initial assessment of the data distribution and identification of potential outliers was performed by reviewing histograms and boxplots and by evaluating the skewness, kurtosis, and Shapiro-Wilk statistics for each reference interval parameter. Outliers were also identified and considered for removal using Dixon's outlier range statistic, employing a criterion of rejection of $D/R > 0.3$.[20] When data were not normally distributed ($p < .05$), the natural log of the values was taken and the data were reassessed.

Normality as evaluated via the Shapiro-Wilk test indicated both left VHS and right VHS parameters were not normally distributed ($p < .001$); transformation (natural log) of the data did not result in a Gaussian distribution ($p < .001$). Calculation of Dixon's Outlier Range Statistic indicated outliers at the lower bound of the data for both left VHS (R = 0.32) and right VHS (R = 0.33). Specifically, values of 8.5 (n = 2 for 1 subject) and 9.0 (n = 6 for 5 subjects) were identified within left VHS and right VHS datasets. Although these were identified statistically as outliers, review of the data found the values to be accurate and appropriate values, thus, they were retained, as recommended in the American Society for Veterinary Clinical Pathology reference interval guidelines.[21] No additional outliers were identified via Dixon's

TABLE 1

Reference Intervals for Left and Right Vertebral Heart Scale (VHS)

Variable	N	Median	Minimum	Maximum	Reference Intervals	
					Lower (90% CI)	Upper (90% CI)
Left VHS	51	10.10	8.70	11.31	8.71 (8.49–8.99)	11.48 (11.24–11.73)
Female Left	24	10.56	9.20	11.31	9.30 (8.87–9.73)	11.75 (11.42–12.03)
Male Left	27	9.74	8.70	10.88	8.49 (8.19–8.85)	11.00 (10.70–11.29)
Right VHS	51	10.30	9.25	11.55	8.98 (8.78–9.21)	11.62 (11.40–11.89)
Female Right	24	10.80	9.50	11.55	9.39 (8.97–9.96)	12.21 (11.85–12.46)
Male Right	27	9.99	9.25	10.80	8.95 (8.75–9.21)	11.03 (10.78–11.25)

CI, confidence interval; VHS, vertebral heart scale.

criteria (left VHS: R = 0.01; right VHS: R = 0.01); however, as the data were not normally distributed, we used robust methods with untransformed data to generate the reference intervals.

For non-Gaussian data, the Wilcoxon Rank Sum test was used to evaluate potential differences in group medians. For the evaluation of paired data, the Mann-Whitney rank sum test was used. Robust (distribution independent) methods were employed to calculate reference intervals and 90% confidence intervals.[22,23] Development of these was conducted according to ASVCP reference interval guidelines.[21]

Spearman rank-order correlations were used to investigate the relationship between left and right VHS and the following echocardiographic parameters (2D LA long axis diameter, M-mode LA diameter, and M-mode LA/Ao ratio).

Inter-observer reliability for left and right VHS were examined by generating the intra-class correlations and 95% confidence intervals where each dog represented a group and each evaluator's measurement was a data point in the respective grouping.

The generation of reference intervals and statistical analyses were conducted using commercially available software.[b,c] A p value $< .05$ was accepted as significant.

Results

Fifty-one normal dachshunds (24 females and 27 males) were evaluated prospectively. The mean age ± SD of the n = 51 animals in our sample was 4.48 ± 3.2 yr and the mean weight was 7.1 ± 2.21 kg (15.62 ± 4.9 lbs). Female dogs had a mean age of 5.8 ± 3.0 yr and mean weight of 6.35 ± 2.0 kg (13.97 ± 4.4 lbs). Males had a mean age of 3.69 ± 3.0 yr and mean weight of 7.77 ± 2.2 kg (17.1 ± 4.9 lbs). One-way analysis of variance revealed males were both younger and heavier than females, $p = .02$.

The median for right lateral VHS was significantly increased compared to the left lateral VHS, 10.3 (range, 9.25–1.55) versus 10.1 (range, 8.70–11.31), ($p < .0001$) via Mann-Whitney rank sum test.

Median left and right lateral VHS for females was 10.56 (range, 9.2–11.31) and 10.8 (range, 9.5–11.55), respectively. Median left and right lateral VHS for males was 9.74 (range, 8.7–10.88) and 9.99 (range, 9.25–10.8), respectively (**Table 1**). Wilcoxon Rank Sum test revealed VHS was significantly larger for females than males in both radiographic views, $p = .0002$.

The mean ± SD 2D LA long axis diameter echocardiographic measurements was 2.36 ± 0.33 centimeters, M-mode LA diameter was 1.61 ± 0.23 centimeters, and M-mode LA/Ao diameter ratio was 1.08 ± 0.15. Male subjects had significantly higher 2D LA long axis diameter measurements (2.48 ± 0.27 versus 2.24 ± 0.36, $p = .01$), but no significant differences were found between genders for M-mode LA diameter measurements or M-mode LA/Ao diameter ratio (Males: 1.66 ± 0.2 versus 1.56 ± 0.25, $p = .10$, and 1.06 ± 0.14 versus 1.11 ± 0.16, $p = .28$, respectively). Spearman rank order correlation coefficients indicated that VHS measurements were not significantly correlated with any of the LA echocardiographic parameters (**Table 2**). Although no murmurs were identified on physical examination at the time of enrolment, 16 dogs were found to have trivial to mild mitral regurgitation on color-flow Doppler. All 2D LA measurements in these subjects were within size-matched reference ranges.[19]

Observers' measurements of VHS indicated a high degree of inter-observer consistency with an intra-class correlation coefficient (95% confidence interval) of 0.95 (0.93–0.96) for both left and right VHS measurements.

Discussion

The dachshund is consistently one of the most popular breeds in America according to the American Kennel Club 2011 Dog Registration Statistics (http://www.akc.org/reg/dogreg_stats.cfm)

TABLE 2

Correlations for Vertebral Heart Scale (VHS) and Echocardiographically Derived Left Atrial Dimensions

	Left VHS			Right VHS		
Variable	**Correlation**	**95% CI**	***P* Value**	**Correlation**	**95% CI**	***P* Value**
2D LA	-0.25	(-0.49–0.02)	.07	-0.25	(-0.49–0.03)	.08
M-mode LA	-0.06	(-0.33–0.22)	.69	-0.05	(-0.32–0.23)	.75
LA/Ao	-0.09	(-0.19–0.36)	.53	-0.09	(-0.19–0.36)	.53

2D, two-dimensional; CI, confidence interval; LA, left atrium; LA/Ao, left atrium to aortic valve diameter ratio; VHS, vertebral heart scale.

and MMVD is the most common heart disease in the breed.[15,24,25] The presence of a left apical systolic murmur due to mitral regurgitation occurs in approximately 50% of dachshunds by 10 yr of age.[15,16,26] Although affected animals may remain asymptomatic for years, congestive heart failure is a common sequela.[17] Recently, VHS has been shown to be a predictive variable for ensuing heart failure.[27]

This study was performed to determine if the breed-specific VHS for the normal dachshund was within the original canine reference range of 9.7 ± 0.5 vertebrae, assess inter-observer variability for measuring VHS, and determine if VHS correlated with echocardiographic measures of LA dimension. The original canine reference range determined by Buchanan and Bucheler was established based on a mixed population of breeds. Since its development, the range has been shown to be inapplicable to several breeds whose normal heart size exceeded these values. In a retrospective study performed in 2001, the boxer (11.6 ± 0.8), Labrador retriever (10.8 ± 0.6), and Cavalier King Charles spaniel (10.6 ± 0.5) were found to have normal VHS above the established range.[3] Additional studies have shown the greyhound (10.5 ± 0.1), beagle (10.3 ± 0.4), whippet (11.3 ± 0.5), pug (10.7 ± 0.9), Pomeranian (10.5 ± 0.9), bulldog (12.7 ± 1.7), and Boston terrier (11.7 ± 1.4) also have larger normal heart sizes.[6,8,12,13] A 2013 retrospective study evaluating VHS in eight canine breeds found the dachshund to have a mean VHS within the original reference range.[13] However, this study suffered the significant bias of only including radiographic reports that were subjectively interpreted as having a normal cardiac size and did not attempt to exclude dogs that were anesthetized or had non-cardiac diseases. These methods may have falsely decreased the mean VHS. In the current study, we prospectively determined dachshunds to be normal based on physical examination and echocardiography. Additionally, no sedatives or anesthetic agents were used for any parts of the study.

This study documented that the normal median VHS for the dachshund was 10.1 on left lateral and 10.3 on right lateral thoracic radiographs, which is above the original mean reference value of 9.7 vertebrae. Although the median VHS is above the canine reference value, some overlap was observed between reference intervals. It was suggested by Buchanan and Bucheler that a VHS of 10.5 should be used as the upper limit for normal heart size.[4] In a more recent study, a VHS value greater than 10.7 was suggested to be a more accurate value to identify cardiomegaly in dogs with cardiac disease compared to normal dogs.[3] In the present study, the upper end of the reference interval for left and right lateral VHS was 11.48 and 11.62, respectively. Ninety-five percent of our measurements were below 11.1, suggesting that a VHS greater than 11 is a clinically useful cut-off for the upper limits of normal in the dachshund.

In this study, sex and recumbency statistically influenced VHS measurements. Females had larger measurements than males in both recumbencies, and the overall combined right lateral VHS for males and females was significantly greater than the left. These results differ from Buchanan and Bucheler, who found recumbency and gender did not influence VHS.[4] Similar to our study, Greco et al. documented VHS is significantly greater in right lateral recumbency; however, they found no influence of gender on VHS.[11] They speculated increased VHS in right lateral recumbency may be due to a greater distance of the heart from the radiographic cassette in comparison to left lateral recumbency. The reason for increased VHS measurements in females compared to males is uncertain. Although there were 16 dogs found to have trivial to mild mitral regurgitation, the majority ($n = 13$) were male. Additionally, all but one affected dog were found to have VHS measurements $\leq$10.5. A potential explanation may be due to the increased age of our females compared to the males. In humans, epicardial fat deposition has been shown to increase with advancing age.[28] If a similar finding also occurs in dogs, the result could be an increasing trend in VHS with age. Further studies are necessary to investigate this possibility.

Inter-observer variability showed substantial agreement for measurements of VHS, indicating the VHS method can be reliably performed by observers with varying degrees of clinical experience.

Similarly, a previous study found almost identical mean VHS values between observers from four experience levels, concluding that VHS is independent of the observer's level of clinical experience.[9]

No significant correlation was found between any LA echocardiographic parameters and VHS measurements. While VHS has been shown to reliably detect global heart enlargement, published studies correlating the relationship between specific chamber enlargement to VHS in dogs are lacking. Left atrial enlargement has prognostic significance in dogs with MMVD as it is reflective of the severity and chronicity of mitral valve regurgitation.[29] A strong correlation was identified between VHS and LA/Ao ($R = 0.90$) in dogs with induced cardiomegaly due to rapid ventricular pacing.[30] Additionally, in cats with heart disease, a positive correlation between VHS and LA size has been shown.[31] Other radiographic indices of LA size have been recently proposed but were not evaluated in this study.[32,33] Additional studies evaluating the relationship of VHS and LA size in dachshunds with varying degrees of MMVD is warranted.

A potential limitation of this study was the decision to measure the VHS to the nearest 0.25 vertebral bodies. The original study established the VHS using measurements to the nearest 0.10 vertebral bodies using calipers. We chose to use 0.25 vertebral bodies as we believe that this method reflects the most common approach used in clinical practice.

Conclusion

The VHS method can be used to aid in the radiographic assessment of the dachshund cardiac silhouette. Use of breed-specific VHS may aid in the early recognition of cardiac abnormalities in asymptomatic dachshunds. Results indicate normal dachshunds have a median VHS above the published generic canine reference range. Based on this study, a VHS greater than 11.0 in the dachshund should be considered strongly supportive of cardiac enlargement. In these cases, further clinical investigation such as echocardiography is appropriate to investigate for the presence of underlying heart disease.

FOOTNOTES

[a] eFilm Workstation; Merge Healthcare, Chicago, Illinois

[b] Reference Value Advisor; Geffre, Concordet, Braun, & Trumel, Toulouse, France

[c] SAS software, Version 9.3; SAS Institute Inc., Cary, North Carolina

REFERENCES

1. Berry CR, Graham JP, Thrall DE. Interpretation Paradigms for the Small Animal Thorax. In: Thrall DE, ed. *Textbook of Veterinary Diagnostic Radiology*. 5th ed. Philadelphia: WB Saunders Co; 2007:480–3.
2. Lamb CR, Tyler M, Boswood A, et al. Assessment of the value of the vertebral heart scale in the radiographic diagnosis of cardiac disease in dogs. *Vet Rec* 2000;146:687–90.
3. Lamb CR, Wikeley H, Boswood A, et al. Use of breed-specific ranges for the vertebral heart scale as an aid to the radiographic diagnosis of cardiac disease in dogs. *Vet Rec* 2001;148:707–11.
4. Buchanan JW, Bucheler J. Vertebral scale system to measure canine heart size in radiographs. *J Am Vet Med Assoc* 1995;206(2):194–9.
5. Sleeper MM, Buchanan JW. Vertebral scale system to measure heart size in growing puppies. *J Am Vet Med Assoc* 2001;219(1):57–9.
6. Marin LM, Brown J, McBrien C, et al. Vertebral Heart Size in Retired Racing Greyhounds. *Vet Radiol Ultrasound* 2007;48(4):332–4.
7. Litster AL, Buchanan JW. Vertebral scale system to measure heart size in radiographs in cats. *J Am Vet Med Assoc* 2000;216(2):210–4.
8. Kraetschmer SK, Ludwig K, Meneses F, et al. Vertebral heart scale in the beagle dog. *J Small Anim Pract* 2008;49:240–3.
9. Hansson K, Haggstrom J, Kvart Clarence, et al. Interobserver Variability of Vertebral Heart Size Measurements in Dogs with Normal and Enlarged Hearts. *Vet Radiol Ultrasound* 2005;46(2):122–30.
10. Guglielmini C, Diana A, Pietra M, et al. Use of the Vertebral Heart Score in Coughing Dogs with Chronic Degenerative Mitral Valve Disease. *J Vet Med Sci* 2009;71(1):9–13.
11. Greco A, Meomartino L, Raiano V, et al. (2008). Effects of left vs. right recumbency on the vertebral heart score in normal dogs. *Vet Radiol Ultrasound* 2008;49(5):454–5.
12. Bavegems V, Caelenberg AV, Duchateau L, et al. Vertebral heart size ranges specific for whippets. *Vet Radiol Ultrasound* 2005;46(5):400–3.
13. Jespen-Grant K, Pollard RE, Johnson LR. Vertebral heart scores in eight dog breeds. *Vet Radiol Ultrasound* 2013;54(1):3–8.
14. Buchanan JW. Prevalence of cardiovascular disorders. In: Fox PR, Sisson D, Moise NS, eds. *Textbook of Canine and Feline Cardiology*. Philadelphia: WB Saunders Co; 1999: 457–70.
15. Olsen LH, Fredholm M, Pedersen HD. Epidemiology and Inheritance of Mitral Valve Prolapse in Dachshunds. *J Vet Intern Med* 1999;13: 448–56.
16. Serfass P, Chetboul V, Sampedrano CC, et al. Retrospective study of 942 small-sized dogs: Prevalence of left apical systolic heart murmur and left-sided heart failure, critical effects of breed and sex. *J Vet Card* 2006; 8:11–8.
17. Borgarelli M, Crosara S, Lamb K, et al. Survival characteristics and prognostic variables of dogs with preclinical chronic degenerative mitral valve disease attributable to myxomatous degeneration. *J Vet Intern Med* 2012;26(1):69–75.
18. Boon JA. Evaluation of size, function, and hemodynamics. In: *Manual of Veterinary Echocardiography*. Ames, IA: Blackwell; 2006: 193–5.
19. Rishniw M and Erb HN. Evaluation of four 2-dimensional echocardiographic methods of assessing left atrial size in dogs. *J Vet Intern Med* 2000;14(4):429–35.
20. Dixon WJ. Processing data for outliers. *Biometrics* 1983;9:74–89.
21. Fredrichs KS, Harr KE, Freeman KE, et al. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. *Vet Clin Pathol* 2012;41(4):441–53
22. Horn PS, Pesce AJ. *Reference intervals: A User's Guide*. Washington, DC: AACC Press; 2005.
23. Horn PS, Pesce AJ, Copeland BE. A robust approach to reference interval estimation and evaluation. *Clin Chem* 1998;44(3):622–31.
24. Thrusfield MV, Aitken C, Darker P. Observations on breed and sex in relation to canine heart valve incompetence. *J Small Anim Pract* 1985; 26(12):709–17.

25. Haggstrom J, Hansson K, Kvart C, et al. Chronic valvular disease in the Cavalier King Charles Spaniel in Sweden. *Vet Rec* 1992;131(24):549–53.
26. Pedersen HD, Kristensen B, Norby B, et al. Echocardiographic study of mitral valve prolapse in Dachshunds. *J Vet Med Ser A* 1996;43(2):103–10.
27. Reynolds CA, Brown DC, Rush JE, et al. Prediction of first onset of congestive heart failure in dogs with degenerative mitral valve disease: The PREDICT cohort study. *J Vet Card* 2012;14:193–202.
28. Silaghi A, Piercecchi-Marti MD, Grino M, et al. Epicardial adipose tissue extent: relationship with age, body fat distribution, and coronaropathy. *Obesity* 2008;16(11):2424–30.
29. Borgarelli M, Savarino P, Crosara S, et al. Survival characteristics and prognostic variables of dogs with mitral regurgitation attributable to myxomatous valve disease. *J Vet Intern Med* 2008;22(1):120–8.
30. Nakayama H, Nakayama T, Hamlin RL. Correlation of cardiac enlargement as assessed by vertebral heart size and echocardiographic and electrocardiographic findings in dogs with evolving cardiomegaly due to rapid ventricular pacing. *J Vet Intern Med* 2001;15:217–21.
31. Guglielmini C, Baron Toaldo M, Poser M, et al. Diagnostic accuracy of the vertebral heart score and other radiographic indices in the detection of cardiac enlargement in cats with different cardiac disorders. *J Feline Med Surg* 2014;16(10):812–25.
32. Le Roux A, Rademacher N, Saelinger C, et al. Value of tracheal bifurcation angle measurement as a radiographic sign of left atrial enlargement in dogs. *Vet Radiol Ultrasound* 2012;53(1):28–33.
33. Sanchez X, Prandi D, Garcia-Guasch L, et al. New radiographic measurements of left atrial size in dogs with degenerative mitral valve disease: preliminary study. *J Vet Intern Med* 2013;27:639.