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Interobserver variability of radiographic methods for the evaluation of left atrial size in dogs

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Abstract

The objectives of this retrospective, observer agreement study were to (a) test vari- ability of radiographic left atrial dimension (RLAD) and vertebral left atrial size (VLAS) measurements among observers with different levels of expertise in thoracic radiology and cardiology, (b) assess whether one method is better than the other in detecting left atrial enlargement (LAE), and (c) assess the agreement among RLAD, VLAS, and Ameri- can College of Veterinary Internal Medicine (ACVIM) classes. Seventy-four dogs (eight healthy and 66 with mitral valve disease) with thoracic radiographs and echocardio- graphy performed on the same day were reviewed. Thirty showed echocardiographic LAE. Left atrial dimension was quantified using RLAD and VLAS by six different oper- ators with three levels of clinical experience in veterinary cardiology/radiology. Verte- bral heart score and fourth thoracic vertebra (T4) were also measured. Differences in T4, vertebral heart score (VHS), RLAD, and VLAS measurements were found among six operators and among the three levels of clinical expertise as well as between veteri- nary cardiology readers and veterinary radiology readers ($P < .05$). The area under the receiver operating characteristic (AUC) curve for VHS showed good performances for all observers and level and type of expertise; the AUC for RLAD and VLAS was subopti- mal only for the radiology student. Our RLAD and VLAS cutoffs (1.9 and 2.43 v, respec- tively) were better related to qualitative radiographic than quantitative echocardio- graphic LAE evaluation. Radiographic LA dimension and VLAS showed an increase pro- portional to the worsening of the ACVIM class. In conclusion, these results allow us to affirm that RLAD and VLAS are reproducible measurements for detecting LAE. Better performances are associated with clinical expertise and background.

KEYWORDS

canine, left atrial dimension, mitral valve disease, X-ray

Abbreviations: 2D, two-dimensional; ACVIM, American College of Veterinary Internal Medicine; AUC, area under the ROC curve; CHF, congestive heart failure; CV, coefficient of variation; CVC, caudal vena cava; IQR, interquartile range; LA, left atrial; LA/Ao, left atrium to aorta ratio; LVIDaN, normalized left ventricular internal diameter in diastole; MMVD, myxomatous mitral valve disease; RLAD, radiographic left atrial dimension; ROC, receiver operating characteristic; T4, fourth thoracic vertebra; VHS, vertebral heart score; VLAS, vertebral left atrial size.

The authors followed STROBE reporting guidelines.

1 | INTRODUCTION

Myxomatous mitral valve disease is the most common acquired cardiac disease in dogs and affects up to 90% of patients with more than 10 years within some small breeds.[1] Typically, the disease has a long subclinical period in which a left apical systolic murmur gradually

The results of this work were never presented or published before.

becomes more evident in relation to the worsening of mitral valve regurgitation.[2] Some dogs affected by myxomatous mitral valve disease (MMVD) develop clinically detectable left atrial enlargement (LAE) and left ventricular dilatation. The magnitude of LAE is consid- ered a reliable indicator of disease severity.[1] Clinical signs of MMVD, such as dyspnea/tachypnea, syncope, and congestive heart failure (CHF), can develop in affected dogs and are generally associated with LAE and the progression of the disease.[3,4] Results of multiple large clinical studies involving dogs with MMVD indicate that echocardio- graphic assessment of left atrial (LA) size predicts the risk for CHF,[5] the timing of the correct introduction of medications prior to the onset of CHF, and the prognosis.[3,6–8] Echocardiographic assessment of LA size is considered an important part of the diagnostic evaluation for dogs with MMVD. Body size varies widely among dogs and for this reason LA size is indexed thanks to several echocardiographic methods.[9,10]

Left atrium to aorta ratio (LA/Ao) in short axis view is one of the most frequently used methods.[11] However, echocardiographic assessment of LA size is not always a practical option for the owners, due to the limited availability and the cost of echocardiographic examination. In addition, the veterinary clinician needs specific training and skills to correctly perform and interpret an echocardiographic examination.

For these reasons, chest radiograph covers an important role in the diagnosis and in the staging of dogs affected by MMVD. Thoracic radiography is therefore recommended as part of the diagnostic eval- uation for all dogs with suspected MMVD regardless of the clinical sign presentation.[12] Compared with echocardiography, thoracic radio- graphy is widely available and cost-effective. However, radiographic assessment of LA size could be primarily subjective and prone to error. Consequently, quantitative methods for radiographic estimation of LA size would be of clinical value, particularly when echocardiog- raphy is not readily available. The LA measurements that have been described are radiographic left atrial dimension (RLAD) and vertebral left atrial size (VLAS).[13,14] However, literature lacks information about the repeatability of these quantitative radiographic parameters of LAE for observers with different levels of clinical expertise. Only Duler et al in 2018 assessed interreader agreement by a panel of readers with dif- ferent level of experience and expertise over a wide range of breeds and different cardiac disease but only qualitative radiographic LAE was evaluated.[13–15] Moreover, there are no published studies comparing the two methods. We hypothesized that RLAD and VLAS would both accurately predict the presence of echocardiographic LAE in a large and varied sample of dogs affected by MMVD and that they would have strong agreement with echocardiography to differentiate dogs with different levels of LAE.

The first aim of this study was to investigate variability of RLAD and VLAS in dogs between observer with different levels of expertise in thoracic radiology and cardiology. A second aim was to assess whether one method is better than the other in detecting LAE. The tertiary aim was to compare obtained cutoffs of LAE with those reported in liter- ature, correlating them with quantitative echocardiographic and qual- itative radiographic LAE evaluation. The last aim of this study was to assess the agreement among RLAD, VLAS, and American College of Veterinary Internal Medicine (ACVIM) classes.

2 | MATERIALS AND METHODS

2.1 | Case selection

This was a retrospective, observer agreement study design. Clinical records of dogs presented to the Cardiology Service of Veterinary Teaching Hospital, (University of Milan) between June 2018 and Octo- ber 2019 were retrieved. Owner consent was obtained for all dogs during the first visit. All dogs, healthy or affected by MMVD, had to have a complete clinical evaluation including physical examination, complete blood count, biochemistry panel, at least one thoracic radio- graphic examination in laterolateral right decubitus, and echocardio- graphic examinations carried out within the same day without chem- ical restraint. The thoracic radiographic studies were obtained with a digital system (RX D-VET G35i, FUJIFILM Italia S.P.A., Milano, Italia) and radiographic exposure factors for each dog were based on patient body size. Clinical and echocardiographic evaluations were carried out by two different well-trained experienced practitioners (MB [PhD vet- erinary cardiology student] and CL [veterinary cardiology clinician with >15 years of clinical and echocardiography experience]). A dog was considered healthy if the medical history and the results of the afore- mentioned procedures did not reveal any alterations. This decision was based on the more experienced observers' consensus (veterinary radi- ology and cardiology specialist clinicians with >15 years of experience in small animal clinical practice). Within the group of healthy subjects, there were no subjects predisposed to the development of MMVD (ACVIM A).

2.2 | Echocardiographic data

Myxomatous mitral valve disease was diagnosed on the basis of a typical systolic mitral regurgitation murmur and echocardiographic findings such as the presence of valvular thickening, irregularity, and prolapse of the mitral valve apparatus observed by two-dimensional (2D) echocardiography and evidence of mitral valve regurgitation during systole as determined by color Doppler examination.[16–18] The following echocardiographic variables were selected for successive statistical evaluation: LA/Ao, normalized left ventricular internal diameter in diastole (LVIDaN), E peak velocity, and E/A waves ratio (E/A). Left atrium to aorta ratio was obtained by 2D transthoracic echocardiography from a right parasternal short axis view as described in earlier reports.[19] The measurement was performed by the most experienced veterinary cardiology reader (CL) in early ventricular diastole using the first frame after aortic ejection, when the Ao appeared as a symmetrical three-leaf clover with closed aortic valves and a teardrop-shaped LA. The investigator was unaware of (blinded to) the radiographic findings while performing the echocardiographic measurements. Dogs with MMVD were allocated to one of four classes in accordance with ACVIM guidelines[12]: the healthy group was free of echocardiographic abnormalities; the stage B1 group had MMVD, LA/Ao < 1.6, and LVIDaN < 1.7; the stage B2 group had MMVD, LA/Ao ≥ 1.6 and LVIDaN ≥ 1.7 without evidence of previous (compensated) or concurrent (decompensated) CHF; and stage C-D group had MMVD and evidence of compensated or decompensated CHF. Decompensated left-sided CHF was diagnosed by the same veterinary cardiology reader (CL) based on radiographic evidence of cardiogenic pulmonary edema and the presence of respiratory diffi- culty that were responsive to furosemide administration. Dogs were assigned to one of two groups according to the absence or presence of

LAE defined by 2D echocardiography as LA/Ao ≥ 1.6.[11]

2.3 | Exclusion criteria

Dogs presenting with radiographic and/or echocardiographic changes compatible with cardiac diseases other than MMVD, such as cardiomy- opathy, myocarditis, congenital heart defects, cardiac tumors, and diag- nosed arrhythmias, were not included in the study. Those subjects that had concomitant diseases that could have reduced heart size (micro- cardia, eg, ongoing Addison's disease or secondary to hypovolemic shock, severe dehydration, or cardiac tamponade) were eliminated from the study. Dogs that were <1-year old (skeletally immature) and those with acute CHF secondary to chordae tendineae rupture were excluded. Thoracic radiographs that revealed overt malposition of the patient (as the considerable rotation, the movement due to excessive breathing for tachypnea, or the brachial muscles superimposition on the cranial aspect of the thorax) or the presence of thoracic vertebral abnormalities (eg, hemivertebrae) were not included in the study.[20] The exclusion from the study, for problems mentioned above related to the radiographic anomalies, was evaluated by the most experienced veterinary radiology reader (veterinary radiology specialist clinician with >15 years of experience).

2.4 | Radiographic measurements

To be included in the study, the radiographs had to be of good tech- nical and diagnostic quality. The basis for the selection of radiographs was to create a test situation such that the results would not be biased by great difficulties in finding the anatomic landmarks, by obliquity or uncommon variations of thoracic conformation. The radiographic mea- surements were performed in all dogs on right lateral thoracic radio- graphs by six different operators with three levels of clinical experi- ence (veterinary students, PhD students, and clinicians with >15 years of experience in small animal clinical practice) in veterinary cardiology or veterinary radiology. The levels of expertise for the observers were described (Table 1). All observers were given both oral and written instructions on how to perform the measurements and written instruc- tions were available throughout their evaluation. In our hospital, unlike the vertebral heart score (VHS) method, RLAD and VLAS were not part of the routine evaluation of thorax radiographs neither for vet- erinary cardiology readers nor for veterinary radiology readers before this study. In addition, the veterinary cardiology student had previously assisted the clinician and the veterinary cardiology PhD student dur- ing their test evaluations of patients not included in the study, whereas the radiology student performed the measurements only basing on oral and written instructions since the first time. Masking and randomiza- tion methods were applied in order to minimize potential biases. Each radiograph was evaluated three times by each observer and mean val- ues were considered for statistical analysis.

First for each patient, by reviewing all thoracic right lateral views each observer determined the presence or absence of LAE (subjective evaluation of LA size). Observers were not provided any supplemental information about how to gauge subjectively the severity of LAE.

For each subject, the fourth thoracic vertebra (T4), the VHS as described by Buchanan,[21,22] RLAD, and VLAS as described by Salguero and Malcolm were measured.[13,14] The same radiological reading work- station (OsiriX MD Dicom Viewer Pixmeo Sarl, version 8.0, Geneva, Switzerland; Apple iMac Retina 5k, 27-inch monitor, 2019—Cupertino, CA, USA) was used for all measurements by the six observers. The long

axis of T4 body, from the cranioventral to the caudoventral border, including its caudal disc was measured in millimeters and used as an indicator of vertebral length in the thoracic spine. The VHS cardiac long axis (L) was measured from the ventral border of the left main stem bronchus (carina) to the more distant point of the cardiac apex. For the purpose of this study, the carina was defined as the radiolucent circular or ovoid structure within the trachea that represented the bifurcation of the left and right mainstem bronchi. To include the LA body, a slight variation was introduced, consisting in measuring the short axis (S) at the level of the dorsal edge of the caudal vena cava (CVC).[23] The software was used to apply a 90-degree angle between L and S. Long and short axes were then repositioned over the thoracic vertebrae from the cranial edge of the T4, parallel to the vertebral column, and each length was then expressed in terms of the number of thoracic vertebrae (v) to the nearest 0.1 v. The sum of the two was used as VHS. In accordance with Buchanan, the vertebral body with its caudal intervertebral disc was considered as a unit. Radiographic LA dimension was obtained from the same radiographic images used for VHS quantification. The computer software was used to ensure a line bisecting the 90° angle formed by the intersection of the VHS L and S axes connecting this point with the radiographic projection of the dorsal edge of the LA (Figure 1). This length was then normalized to vertebral unit (v) starting at the cranial edge of T4 and to the nearest 0.1 v and used as RLAD. In cases where it was difficult to differentiate the dorsal anatomical boundaries of the LA and the neighboring pulmonary veins, the most dorsal aspect of the soft tissue opacity seen at this level was routinely used for all measurements. The VLAS was measured as a line drawn from the middle of the most ventral aspect of the carina to the most caudal aspect of the LA where it intersected with the dorsal border of the CVC. Like the VHS method,[23] a second line that was equal in length to the first was drawn beginning at the cranial edge of T4 and extending caudally just ventral and parallel to the verte- bral canal (Figure 2). The VLAS was defined as the length of the second line expressed in vertebral body units to the nearest 0.1 vertebra.

2.5 | Intra- and interobserver variability for fourth thoracic vertebra, vertebral heart score, radiographic left atrial dimension, and vertebral left atrial size

Intraobserver agreement was studied using a complementary pilot study in which two different observers (the two PhD students) mea- sured T4, VHS, RLAD, and VLAS in radiographs from 10 dogs randomly chosen. Each set of masked and randomized radiographs was evaluated in three occasions. Intraobserver variability was measured as the mean variability between replications for the same animal.

determine interobserver agreement of T4, VHS, RLAD, and VLAS measurements, all six investigators individually To perform the measurements for the same 74 dogs. All investigators were blinded to the clini- cal diagnosis for each dog and measurements determined by the other investigators. Interobserver variability was calculated also between types and levels of clinical expertise. Interobserver coefficient of vari- ation (CV) was measured as the variability of the mean difference for each animal and the first measurement of each observer was paired to the first measurement of the others.[24]

Coefficients of variation of T4, VHS, RLAD, and VLAS were eval- uated also among veterinary cardiology readers and veterinary radi- ology readers and for different levels of clinical expertise for all 74 included dogs on right lateral thoracic radiographic images.

2.6 | Statistical analysis

Statistical analyses were performed by a veterinary cardiology specialist clinician (CL) with statistics training using commercially available statistics software (SPSS 26.0, IBM, SPSS, USA; MedCalc Statistical Software version 19.3.1, MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2020). Descriptive statistics were generated. The distribution of data for continuous variables was assessed for normality by means of the Kolmogorov-Smirnov test. None of the variables were normally distributed, and results were reported as the median and interquartile range (IQR) (25th to 75th percentile) unless otherwise specified. Continuous variables were compared among the observer's groups by Friedman's chi square test and sign test for Wilcoxon rank and among the ACVIM classes was performed the Kruskal-Wallis test. The Bonferroni correction post hoc test was executed for multiple comparisons. The Spearman rank-order correlation coefficient (r_S) was calculated to determine the strength of the association between VHS, RLAD, VLAS, and LA/Ao and LVIDaN. The correlation was considered weak, moderate, strong, or perfect, respectively, when the value of the correlation coefficient was .1-.3, .4-.6, .7-.9, or 1.[25] Receiver operating characteristic (ROC) analysis was used to assess the optimal cutoff value for RLAD and VLAS to predict LAE as determined on the basis of quantitative echocardiographic criterion (LA/Ao ≥ 1.6). This analysis was performed for each observer and for all them together, and then also for level and type of expertise. The optimal clinically relevant cutoff value for VHS, RLAD, and VLAS was determined using the Youden index. The area under the ROC curve (AUC) was used to assess the diagnostic accuracy and quantify the predictive value of RLAD and VLAS: AUC = 0.5, the test is not informative; 0.5 < AUC ≤ 0.7, the test is inaccurate; 0.7 < AUC ≤ 0.9,the test is moderately accurate; 0.9 < AUC < 1.0, the test is highly accurate; AUC = 1, perfect test.[26] Areas under the curves were compared using Delong's method.[27] Furthermore, Cohen's kappa statistic measured the reliability and the agreement between two different methods in the evaluation of LAE. In particular, the cutoffs of RLAD and VLAS reported by literature and those obtained in this study were compared with the objective (echocardiographic) and subjective (radiographic) evaluation of LAE (presence/absence of LAE). Kappa agreement (K [95% confidence interval]) was interpreted as slight agreement (0.01-0.2), fair (0.21-0.40), moderate (0.41-0.60), substantial (0.61-0.80), and almost perfect agreement (0.81-0.99).[28]

P-values < .05 were considered significant for all analyses.

3 RESULTS

3.1 | Dogs

The study population included 74 dogs, 39 males (52.7%; n = 26 [35.1%] intact males and n = 13 [17.6%] neutered males) and 35 females (47.3%; n = 7 [9.5%] intact females and n = 28 [37.8%] neutered females), with a median age of 10.89 (IQR = 8.56-12.43) years and a median weight of 9.10 (IQR = 6.10-16.49) kg. The pop- ulation was composed by 26 mongrels (35.1%), 15 Cavalier King Charles Spaniel (20.3%), seven Chihuahua (9.5%), four Jack Russel Ter- rier (5.4%), three Dachshund (4.1%), three English Setter (4.1%), two Labrador Retriever (2.7%), two Schnauzer (2.7%), and other breeds in lower percentage. Eight dogs were healthy (10.8%) and 66 (91.9%) were affected by MMVD at different stage (n = 22 [29.7%], B1; n = 16 [21.6%], B2; n = 25 [33.8%], C; n = 3 [4.1%], D). Thirty dogs (40.5%) did not present LAE and 44 (59.5%) had LAE. Ten dogs (22.7%) with LAE presented pulmonary edema. The sex distribution, the age, and the weight did not differ among the two groups (*P*-values > .05).

Clinical, echocardiographic, and radiographic data for each ACVIM class were summarized (Supporting Information 1). The LA size for dogs with LAE was greater than dogs without LAE regardless of the method (VHS, RLAD, or VLAS) used to assess LA size (*P*-values < .01).

3.2 | Variability of radiographic measurements between observers

Measurements of T4, VHS, RLAD, and VLAS were obtained for all subjects, including dogs presenting pulmonary edema. For all sub- jects, there were differences between cardiology and veterinary radiology readers for T4, VHS, RLAD, and VLAS (*P*- values < .001). For all radiographic measurements, there were differences between clinicians with >15 years of experience and both veterinary students and PhD students (*P*-values < .01). The analysis carried out on the individual observers showed that there were differences for T4 and VLAS measurements between the veterinary cardiology specialist with higher level of expertise (observer 1) and all the other observers (*P*-values < .05).

Differences between observers for VHS and RLAD and median and IQRs of the radiographic data for each observer (Table 2) and each type and level of expertise for the whole population were summarized (Table 3). Regarding the T4, observer 2 (PhD veterinary cardiology student) demonstrated a 0.02 v variability between measurements, whereas observer 5's (PhD veterinary radiology student) was 0.01 v. Regard- ing VHS, observer 2 demonstrated a 0.08 v variability between mea- surements, whereas observer 5's was 0.1 v. Regarding RLAD, both observers demonstrated a 0.05 v variability between measurements, whereas for VLAS observer 2 had a variability of 0.03 v and observer 5 of 0.06 v. Intraobserver CV are reported in Table 4.

Interobserver CV of T4, VHS, RLAD, and VLAS between veterinary cardiology and veterinary radiology readers was 0.01, 0.02, 0.09, and 0.03, respectively. Between different levels of clinical expertise (high, moderate, and low), interobserver CV was 0.01, 0.01, 0.08, and 0.04 respectively. Interobserver CV among all six observers of 0.27, 0.08, 0.26, and 0.21 was found for T4, VHS, RLAD, and VLAS measurements. Coefficients of variation among different types and levels of clinical expertise are reported in Table 5.

3.3 Correlation analyses

A strong positive correlation was observed between LA/Ao and VHS; moderate and positive correlations were observed between LA/Ao and RLAD and VLAS. A moderate and positive correlations was observed between LVIDaN and VHS, RLAD, and VLAS (Table 6).

3.4 | Diagnostic accuracy of radiographic left atrial dimension and vertebral left atrial size for predicting echocardiographic left atrial enlargement

The AUC of VHS, RLAD, and VLAS had no differences (*P*-values > .05). The diagnostic accuracy of optimal VHS, RLAD, and VLAS cutoffs for prediction of echocardiographic LAE was reported for all observers, levels, and type of clinical expertise, as well as sensitivity and specificity of each method (Tables 7 and 8).

Receiver operating characteristic curves comparison showed for VHS only a difference between veterinary cardiology reader student (observer 3) and veterinary radiology reader with the higher level of expertise (observer 4) (*P*-values = .043), whereas no differences were observed between veterinary cardiology readers and veterinary radi- ology readers and between different levels of clinical expertise. For RLAD method, there were differences between veterinary radiology student reader (observer 6) and all the veterinary cardiology readers (observers 1, 2, and 3) (*P*-values < .05) and between veterinary car- diology readers and veterinary radiology readers (*P*-values = .007). Nonstatistical differences were observed for RLAD among different levels of clinical expertise. Regarding VLAS method, differences were observed between radiology student reader (observer 6) and all the other observers (*P*-values < .01) and between students and PhD stu- dents (*P*-values = .008). No statistical differences were found between veterinary cardiology readers and veterinary radiology readers for VLAS.

3.5 Comparison between radiographic left atrial dimension and vertebral left atrial size and quantitative echocardiographic and qualitative radiographic left atrial evaluation (yes/no)

The agreement between (Cohen's K coefficients) LAE based on pro- posed/our cutoff of RLAD, VLAS, and qualitative radiographic or quan- titative echocardiographic LAE (LA/Ao ≥ 1.6) was evaluated (Table 8). Both methods (RLAD and VLAS) had higher agreement with the qual- itative radiographic LAE evaluation compared to echocardiographic evaluation and VLAS had higher Cohen's K coefficient values than RLAD (Table 9).

3.5 American College of Veterinary Internal Medicine classes

The radiographic measurements VHS, RLAD, and VLAS increased pro- portionally to ACVIM classes: more severe MMVD (more advanced ACVIM class) were related to higer values of VHS, RLAD, and VLAS (Supplement 1).

4 DISCUSSION

This is the first published study in which new radiographic methods for the assessment of LA size (RLAD and VLAS) were evaluated by observers with different levels and type of clinical expertise. Thoracic radiographs are an easy-to-use diagnostic tool in most veterinary clin- ics and did not represent an excessive cost to the owner. This study allowed us to highlight how a method used for a long time and well- settled in the background of all observers, such as VHS, is reproducible among readers with various types and levels of clinical expertise. The same cannot be said for RLAD and VLAS, as they present slight dif- ferences between veterinary cardiology readers and veterinary radiol- ogy readers (RLAD) and between students and more experienced prac- titioners, such as the PhD students (VLAS). It should be highlighted that it is unknown if the observed differences between observers for these new methods are clinically relevant. With our results we want to emphasize how specific background—in particular, in veterinary cardi- ology with proper training—helps to improve performance and allows even inexperienced observers to obtain reliable measurements with both methods, comparable to those of more experienced clinicians. Different results between students are probably related to the spe- cific background of the veterinary cardiology reader one. Indeed, the veterinary cardiology reader student had a more focused formation on the thorax and on the evaluation of the cardiocirculatory system, whereas the veterinary radiology student reader had a wider training on many other aspects of radiology (thoracic and not), but probably the main difference was related to training in these specific methods for the veterinary cardiology reader one. In this study, six observers rep- resented three levels of expertise; the observers were further divided according to their specialty (veterinary cardiology and veterinary radi- ology). This is the major distinction between the present study and those reported by literature using VHS and radiographic method for the quantitative evaluation of LA size.[13,14,22] Only one study assessed the interreader agreement of subjective radiographic LAE compared to echocardiographic LA evaluation.[12] In our study, analyzing the clinical expertise based on specialty (veterinary cardiology vs veterinary radi- ology) there was a difference between the two groups. Veterinary car- diology readers' values of VHS, RLAD, and VLAS were different to vet- erinary radiology readers (Table 4). Moreover, RLAD's AUC curves dif- fered between the two type of clinical expertise. This may depend on their different sensibility in the identification of the dorsal margin of the left atrium, sometimes superimposed on other structures, such as bronchial tree and the neighboring pulmonary veins. Despite this, CV for these two methods is overlapping for the different types and lev- els of experience, as well as consistent and slightly lower than those reported by literature.[13,14]

The AUC as the sensitivity and the specificity obtained for VLAS in this study was comparable with that reported by Malcolm et al[14] (82.2% vs 84% for AUC; 66% vs 67% for sensitivity; and 88% vs 84% for specificity). On the contrary, lowest values were observed among obtained AUC, sensitivity, and specificity for RLAD compared to those reported by literature (80.8% vs 96.9% for AUC; 71% vs 93.5% for sen- sitivity; and 82% vs 96.8% for specificity respectively). This was prob- ably related to the high level of expertise of the two observers in the study by Salguero et al.[13]

A secondary goal of this study was to analyze RLAD and VLAS in order to identify the best method (with better sensitivity and speci- ficity) for the identification of LAE. Cardiac radiology has been shown to be especially reliable to detect LAE and until recently, there were no objective radiographic measurements (such as VHS to detect car- diomegaly) able to detect and quantify it.[3,29] The results of the statisti- cal analysis showed that RLAD and VLAS were more specific than sen- sitive. This means that, as reported by Malcolm et al[14] and Salguero et al,[13] both methods are able to better identify the probability that a healthy dog does not have LAE. The obtained cutoffs (1.9 and 2.43 v, respectively) were similar to those proposed by the literature (1.8 and 2.3 v),[13,14] even with different levels of expertise, and had a discrete correlation with LA/Ao ($0.4 < K < 0.6$). Our analysis suggests that both methods are good techniques for measuring LAE: showing compara- ble ROC results, slight better K coefficients of agreement with radio- graphic LAE were otherwise observed for VLAS. Probably VLAS was not affected by the presence of pulmonary edema and by the increased opacity in the hilar region, features that make this measurement eas- ier and more repeatable. In addition, the reference points for the VLAS method are easier to identify: sometimes it can be difficult to accu- rately differentiate the dorsal edge of the LA due to the superimposition of the bronchial tree and the neighboring pulmonary veins.[13] The differences for the observer with higher expertise in veterinary cardiol- ogy found in the VLAS could instead depend on the morphology of the bronchial carina, easily recognizable structure, but with an oval shape, which did not always allow a precise identification of the point from which to start the measurement.

Obtained Cohen's K coefficients showed that VLAS and RLAD were better related to qualitative radiographic (LAE yes/no) than quantita- tive echocardiographic LAE evaluation. The obtained optimal cutoff values for RLAD and VLAS to detect LAE (1.9 and 2.4 v, respectively) had a slightly higher K agreement with both radiographic and echocar- diographic LAE evaluation than those reported by Salguero et al and Malcolm et al.[11,14] The last aim of this study was to investigate the agreement among RLAD, VLAS, and ACVIM classes of MMVD. The staging of the radiographic measurements RLAD and VLAS within the ACVIM classes showed that the severity of MMVD increases directly proportional to the radiographic measurements. According to Malcolm,[14] this high- lights that the radiographic methods RLAD and VLAS show an increase in the magnification of the LA proportional to the worsening of the dis- ease. This makes both radiographic methods useful tools for MMVD staging, especially when echocardiographic examination is not avail- able, although it remains the first-choice method for the diagnosis and an accurate staging of the disease. It should be noted that it is not pos- sible to make any therapeutic decision on the basis of this correlation results.

Another aspect to consider in this study was that we decided to use the same reference points described by Buchanan, Salguero, and Malcolm.[11,14,23] Our study differs from a study by Buchanan where the short axis was measured caudal to the tracheal bifurcation to be sure of including LA enlargement.[23] This choice was made because the LA was evaluated in a specific way with other radiographic methods.[13,14]

A potential source of variation among observers was differences in the selection of measurement points.

The AUC of VHS did not present differences between different types and levels of expertise. In addition, the VHS coefficients of varia- tion were lower than the other methods. The only difference found was between the ROC curves of the veterinary cardiology reader student and the veterinary radiology reader with the higher level of clinical expertise. This contrasts what reported by Lamb et al,[30] demonstrating that VHS is a solid method, known by all operators and with easily identifiable reference points. This underlines that the VHS method is extremely reproducible thanks to the familiarity acquired over the years by clinicians and has simple and reproducible reference points even for beginners. The mean VHS value obtained in this study for normal dogs was 10.7 ± 0.65 v, which is slightly above the suggested upper limit for normal heart size in most breeds.[22] However, our sample was very small. Normal value for VHS greater than 9.7 ± 5 v is previously reported by literature.[22,31] In fact, obtained VHS value was greater than the first historically proposed by Buchanan,[22,23] but it is in accordance with successive reports of Jepsen-Grant et al.[31] The number, and hence the contribution, of each breed within the 74 dogs that were used to derive that mean was not homogeneous, and this may have influenced the obtained values.[22,31] The use of breed- specific VHS values is needed for the VHS method to have a higher specificity for normal heart size. In this study, due to breed variability of subjects, it was not possible to apply a breed-specific VHS value. When comparing the VHS range for all observations and the different groups of observers, there was an overlap between normal and slight cardiomegaly but no overlap between slight and moderate cardiomegaly. As reported in literature,[32] this is to be expected as dogs classified as slight cardiomegaly only had an increased cardiac width and no dorsal displacement of the trachea. Dogs classified to have moderate and severe cardiomegaly had an increase in both long and short axis dimensions.

In our study, one vertebral unit consists of both the length of the vertebrae and its caudal disc, following Buchanan's original study for each whole number of vertebrae and not for the decimal number. Addi- tionally, we wanted to clearly define the vertebral measurement points and instructed the observers to use the cranioventral and caudoventral borders. This clarification was needed because of variations in shape of the cranial vertebral endplate. Some dogs have a pronounced con- cavity in the mid part of the endplate, both cranial and caudal. If some observers used the mid part and some the ventral part, there would be a difference in measurement data based on anatomy and not experi- ence level.

The identification of the reference points could justify the observed differences for the measurements of T4, RLAD, and VLAS. In particu- lar, regarding T4, the more experienced veterinary cardiology reader tended to underestimate the measurement by not properly including the caudal disc, probably because of the habit of not considering it.

The present study was not without limitations. A short period of inclusion was chosen; dogs with cardiac condition other than MMVD and dogs with echocardiography and thoracic radiograph examination performed on different days were excluded. These strict inclusion/exclusion criteria contributed to reduce the size of our pop- ulation. Furthermore, because the inclusion was based on the clinical findings, it did not allow the creation of homogeneous study groups; this constitutes a common limit to clinical research. For this reason, the number of dogs with pulmonary edema that met all the inclusion criteria was limited and this did not allow us to state with certainty that both methods are adequate also in this clinical condition. The breed variable could not be considered for RLAD and VLAS methods due to the large number of mixed breeds included. Another limita- tion is that no other echocardiographic methods, other than LA/Ao, have been used for the assessment of LA dimensions in short axis view.[9,10,14] Although a severe quality control of thoracic radiograph was guaranteed, there was also a possible variability due to a minimum rotation of the thorax. Finally, due to the retrospective nature of this work, not all dogs included had a complete chest radiographic study with three projections. The authors emphasize, however, that the correct evaluation of the chest includes at least three radiographic projections and not only the right lateral view described in this study.

In conclusion, results of the present study indicate that new radio- graphic measurements RLAD and VLAS demonstrated high sensitiv- ity and specificity for detecting LAE with a moderate/strong correla- tion with LA/Ao ratio and especially with qualitative radiographic LAE evaluation in dogs affected by MMVD at different ACVIM stages. Tho- racic radiographs and evaluation of LA dimension are a simple and cost- effective tool that can be used when echocardiography is not available, also by low experienced practitioners. Better performances are associ- ated with increased clinical expertise and background.

LIST OF AUTHOR CONTRIBUTIONS

Category 1

(a) Conception and Design: Locatelli, Bagardi, Brambilla (b) Analysis of Data: Locatelli, Bagardi, Manfredi, Zani (c) Interpretation of Data: Locatelli, Bagardi

Category 2

(a) Drafting the Article: Bagardi, Locatelli, Manfredi, Zani
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Category 3

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Additional supporting information may be found online in the Support- ing Information section at the end of the article.