Assessment of left atrial volume in dogs

Citation for published version:
Bouvard, J, Thierry, F, Culshaw, G, Schwarz, T, Handel, I & Martinez-Pereira, Y 2019, 'Assessment of left atrial volume in dogs: comparisons of two-dimensional and real-time three-dimensional echocardiography with ECG-gated multidetector computed tomography angiography', *Journal of Veterinary Cardiology*. https://doi.org/10.1016/j.jvc.2019.06.004

Digital Object Identifier (DOI):
10.1016/j.jvc.2019.06.004

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
Journal of Veterinary Cardiology

Manuscript Details

Manuscript number	JVC_2018_115_R4
Title	Assessment of left atrial volume in dogs: comparisons of two-dimensional and real-time three-dimensional echocardiography with ECG-gated multidetector computed tomography angiography.
Article type	Clinical Studies

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Keywords	canine; cardiac volumes; biplane modified Simpson method; biplane area-length method; advanced cardiac imaging

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2nd June 2019

Dear Dr Fonfara,
Editor-in-Chief
Journal of Veterinary Cardiology

Re: Invitation to revise manuscript JVC_2018_115, Assessment of left atrial volume in dogs: comparisons of two-dimensional and real-time three-dimensional echocardiography with ECG-gated multidetector computed tomography angiography.

Thank you again for conditionally accepting our manuscript.
My apologies for the missed editorial changes. I reviewed the complete manuscript and have had it proof read by a colleague before resubmission. I hope that it will be satisfactory to process the manuscript further.

Thank you very much for your kind feedback and your consideration of this resubmitted manuscript.

Yours sincerely,

Dr. Jonathan Bouvard

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Assessment of left atrial volume in dogs: comparisons of two-dimensional and real-time three-dimensional echocardiography with ECG-gated multidetector computed tomography angiography

Short title: Two-dimensional and three-dimensional estimations of left atrial volume in dogs

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Results were presented at the 28th congress of the European College of Veterinary Internal Medicine Companion Animals, Rotterdam, Netherlands, 2018.

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Abbreviations

2D	two-dimensional
3D	three-dimensional
ALM	area-length method
CI	confidence intervals
ECG	electrocardiographic
ICC	intraclass correlation coefficient
LA	left atrium
LAV	left atrial volume
LAV_{max}	maximum left atrial volume
LAV_{min}	minimum left atrial volume
LoA	limits of agreement
MDCTA	multi-detector computed tomography angiography
MOD	method of disk
RT-3DE	real-time three-dimensional echocardiography

Introduction

Left atrial size is a clinically useful measurement in left-sided cardiac disease, and is acknowledged as a predictor of morbidity and mortality in human and veterinary medicine [1–5]. Left atrial size is routinely measured clinically with two-dimensional (2D) transthoracic echocardiography at ventricular end-systole or early diastole, and indexed to aortic root (short axis) [6–8]. However, the left atrium (LA) is a three-dimensional (3D) structure with complex geometry, and because LA enlargement may occur non-uniformly in a number of directions (cranio-caudal, medio-lateral, and ventro-dorsal) [9,10], uniplanar assessment of its size may be unreliable. It has been suggested that measurement of left atrial volume (LAV) on 2D echocardiography reduces these errors [11]. The American Society of Echocardiography recommends calculating LAV either by the disc summation algorithm (method of disk {MOD}), which is similar to the method for measuring left ventricular volume, or by an ellipsoid geometric model using the LA areas and lengths (area-length method {ALM}). Both techniques require two non-foreshortened left apical orthogonal views (two-chamber and four-chamber views) and are dependent on mathematical assumptions based on the LA having a fixed shaped. Consequently, they systematically underestimate LAV in people [12–15]. This has been overcome by the advent of new 3D imaging modalities such as real-time 3D echocardiography (RT-3DE) [16–20], electrocardiographic (ECG)-gated multi-detector computed tomography angiography (MDCTA) [13,21–25] and cardiac magnetic resonance imaging [12,25–27]. Left atrial volume obtained by 2D echocardiography offers limited accuracy. This is due to its reliance on geometric assumptions and the dependency on the view. To avoid LA foreshortening, two-chamber and four-chamber views should be truly orthogonal and optimised to maximise LA length and base in each view. Unlike 2D echocardiography, RT-3DE includes the entire LA within the 3D volume dataset and therefore is not subject to foreshortening, eliminates the limitations of geometric assumptions and thus reduces errors in volumetric measurement [10].

Furthermore, RT-3DE has good spatial and temporal resolution and, in people, has a greater correlation than its 2D counterpart with both ECG-gated cardiac magnetic resonance imaging and MDCTA, the current gold standards in volumetric measurement [12,24,25,27]. The introduction of new RT-3D transducers has resulted in sufficiently high frame rate image acquisition for veterinary clinical applications. Equally, post-processing analysis software using semi-automated border detection algorithms minimizes the initial learning curve and facilitates analysis [28,29]. However, to date, RT-3DE assessment of LAV has only been validated in veterinary cardiology in one study including six healthy dogs [30].

We hypothesised that RT-3DE is more accurate than 2D echocardiography at calculating canine LAV. To investigate this hypothesis, we compared maximum LAV (LAV_{max}) and minimum LAV (LAV_{min}), measured by biplane ALM, biplane MOD and RT-3DE, with 64-slice ECG-gated MDCTA in dogs. Correlations between data sets were calculated and intra- and inter-observer variability in these methods were also determined.

Animals, materials and methods

Enrolment

This was a prospective study approved by our local ethics committee (Approval 45.17) and conducted at the Royal (Dick) School of Veterinary Studies between June 2017 – December 2017. Client-owned dogs were considered for enrolment if they had no history of cardiovascular disease and had been referred and scheduled for thoracic MDCTA under general anaesthesia. Informed client consent was obtained for every dog. Dogs were excluded if a murmur, arrhythmia or gallop rhythm was auscultated on physical examination. Dogs underwent oscillometric systolic blood pressure measurement[a], blood sampling for routine haematology and serum biochemistry, and were anaesthetised using a standard protocol determined by a board-certified anaesthetist based on individual patient requirements. Premedicants included acepromazine[b], partial[c] or full[d] opioid agonists and dexmedetomidine[e]. Anaesthesia was induced by intravenous propofol[f], and maintained following endotracheal intubation by isoflurane[g] in oxygen. Electrocardiographic-gated MDCTA was performed, followed immediately by transthoracic echocardiography. Dogs were further excluded if a thoracic mass was seen to compress or displace the heart on ECG-gated MDCTA, or if congenital or acquired cardiac disease was detected on echocardiography. Mild valvular insufficiencies, in the absence of chamber enlargement, were permitted.

Echocardiography

Echocardiography[h] was performed under general anaesthesia by a single resident in cardiopulmonary medicine (JB), under the supervision of a board-certified cardiologist. Images were obtained with 4V-D (1.5 – 4.0 MHz)[i] and M5S-D (1.5 – 4.6 MHz)[j] matrix phased-array transducers during continuous ECG recording. Dogs were positioned alternatively in right and left lateral recumbency for transthoracic 2D, M-mode, pulsed-wave and continuous wave Doppler studies, and RT-3DE in accordance with the recommendations of the Echocardiography Committee of the Specialty of Cardiology of the American College of Veterinary Internal Medicine [31] and the American Society of Echocardiography [10,32]. Images were digitally stored and analysed offline with a commercially available software[k].

Electrocardiographic-gated multidetector computed tomography angiography image acquisition

All MDCTA examinations were performed using a helical acquisition on a 64-slice MDCTA scanner[l]. A transverse plane examination of the thorax was performed using 2 mm slice thickness and 1mm reconstruction interval, medium frequency reconstruction kernel, 80 kVp, 200 mA, 0.35 seconds tube rotation time and a collimator pitch of 0.51. Scan tube current was modulated by an automatic exposure control system[m]. A pre-monitoring transverse scan was set at the level of the aortic arch, and the automated bolus-tracking was set at 100 Hounsfield units with a cycle time of 3 seconds. A retrospectively ECG-gated cardiac MDCTA was performed using 700 mg I/kg of iodinated contrast medium[n] followed by saline flush administered from a dual barrel injector[o] at 5 mL/s and 325 psi. Scan parameters used for the retrospectively gated scan were set to 1.25 mm slice thickness, 0.625 mm spacing between slices, medium frequency reconstruction algorithm, field of view of 12 cm centred over the heart, 80 kVp, 400 mA, 0.35 seconds tube rotation time and collimator pitch of 0.24.

Measurement of left atrial size

Bi-dimensional echocardiography

Angles of simultaneous multiplane 2D were adjusted to obtain left apical four- and two-chamber views that maximised LA size to avoid foreshortening. Images were optimised for endocardial visualisation and temporal and spatial resolution using the focus, depth, sector size, frame rate, gain, compression, and time-gain compensation controls. All measurements were performed at end-diastole (the frame just after mitral valve closure) and end-systole (just before mitral valve opening) to obtain LA_{min} and LA_{max} respectively, according to American Society of Echocardiography guidelines [10]. Care was taken to encompass the entire LA cavity in the data sets. The endocardial borders were traced manually while excluding the left auricular appendage and the ostia of pulmonary veins, if visible (Fig. 1). The mitral annulus was considered as the left atrioventricular border. Three measurements were obtained and averaged for every dog.

Left atrial volume was calculated with the following formulae:

1) Area-length method calculation: $\mathrm{LAV} = \frac{8}{3}\pi \frac{(A4c\ x\ A2c)}{Lmin}$

where A_{4c} is the area in the apical four-chamber view, A_{2c} is the area in the apical two-chamber view, and L_{min} is the shorter length of the LA measured from the centre of the mitral annular plane to the superior border of the chamber.

2) Method of disk calculation: $\mathrm{LAV} = \frac{\pi}{4}\sum_{i=1}^{n}(\mathrm{Di}^{4C})\ (\mathrm{Di}^{2c})\frac{L}{n}hi$

where h_i is the height of the stacked discs *I*, and D_{i4c} and D_{i2c} are two orthogonal minor and major axes derived from four-chamber and two-chamber views respectively.

Real-time three-dimensional echocardiography

Three-dimensional cine loops were acquired over eight consecutive cardiac cycles from the left parasternal view to obtain a single beat full-volume dataset with an average frame rate of 36.6 ± 10.3 frames per second (range 22.4 – 63 frames per second). Loops were digitally stored and analysed offline with commercially available software[k,p]. An effort was made to optimise image quality, increasing the frame rate by narrowing the sector width and decreasing the depth. Maximum left atrial volume and LAV_{min} were derived from semi-automated tracing of the left atrial endocardium at end ventricular systole and diastole respectively. The software[k,p] created an endocardial cast of the left atrium, according to reference points on the hinge-point of the septal leaflet, the hinge-points of the lateral leaflet, and the dorsal border of the left atrium (Fig. 2, Supplemental video I, video available in Supplemental Material online). In all cases, the cast was manually adjusted so that the plane of the mitral valve defined one border, and the visual defect at the entry of the pulmonary veins was bridged. Care was taken to approximate the entire LA endocardial border frame by frame through the cardiac cycle. The pulmonary veins and left auricular appendage were not included. Three measurements were obtained and averaged for each dog from three representative cardiac cycles.

Electrocardiographic-gated multidetector computed tomography angiography

All assessments and measurements were performed by a diagnostic imaging resident (FT) under board-certified veterinary radiologist (TS) supervision, using dedicated DICOM viewer software[q]. A window width of 1700 Hounsfield units and a window level of 200 Hounsfield units were used. The frames which depicted the LAV_{min} (just after mitral valve closure) and LAV_{max} (just before mitral valve opening) were subjectively determined on dynamic series using a 4D CINE mode review[q]. This mode allows dynamic evaluation of a 3D image over time. Atrial volume analysis was performed by drawing regions of interest, delineating the LA border. Initially, two slices were drawn manually at each extremity of the LA, and the operator then drew slices between them at random locations. Subsequently, the software generated several interconnecting regions of interest (semi-automatic segmentation) that were adjusted manually to ensure accuracy. Finally, the software calculated LAV by summing the volumes of all the selected regions. The pulmonary veins and left auricular appendage were not included. The anatomical limits of these structures were subjectively chosen by the operator based on the contour of the LA as part of the semi-automatic segmentation method (Fig. 3).

Variability analysis

To calculate intra-observer within day and between day variabilities, six echocardiograms and six MDCTA studies were randomly selected. Maximum left atrial volume and LAV_{min} were measured by every method (ALM, MOD, 3D volume derived from RT-3DE and ECG-gated MDCTA). The same cardiac cycle from the same cine loops of every echocardiogram and every ECG-gated MDCTA were subjected to repeated analyses by the same investigator (JB and FT respectively) at two different time points on a given day (morning and afternoon) and on two different days.

The same images were used to evaluate inter-observer variability. Two independent observers (YMP for echocardiography and JB for MDCTA) performed independent, blinded and repeated analyses.

Statistical analysis

A sample size of 20 observations per group was estimated to give > 80% power to detect a correlation of at least 0.60 (with a critical p-value of 0.05) [33].

Statistical analyses were performed using commercially available software[r,s,t]. Data were assessed for normality graphically and by use of the Shapiro-Wilk normality test. Continuous variables were reported as mean ± standard deviation. Correlations (r) between echocardiographic techniques and MDCTA were determined by Pearson's correlation test, and defined as excellent ($r \geq 0.90$), very good ($0.90 > r \geq 0.70$), good ($0.70 > r \geq 0.50$) or poor ($r < 0.50$) [34]. For sets of pairs of values, limits of agreement (LoA) and bias were assessed by Bland-Altman plots[r], to visualise the comparisons graphically. Analysis of agreement was performed using intraclass correlation coefficient (ICC), assessing consistency of single measurement with a two-way model (as all methods were used on all cases) and corresponding 95% confidence intervals (CI), where 0 – 0.20 indicates poor agreement, 0.21 – 0.40 indicates fair agreement, 0.41 – 0.60 indicates moderate agreement, 0.61 – 0.80 indicates substantial agreement, and > 0.80 indicates almost perfect agreement. These arbitrary cut-offs were similar to those used by Landis and Koch [35]. A p-value of less than 0.05 was considered to be statistically significant. Coefficients of variation were defined as the standard deviation of the differences divided by the mean of the variable under consideration, and were expressed as a percentage. Values <15% were considered adequate for clinical use [36].

Results

The study population consisted of 20 dogs of various breeds including cross breed (n = 4), Labrador retriever (n = 3), golden retriever (n = 3), cocker spaniel (n = 2), and one of each of the following breeds: flat coat retriever, schnauzer, rough collie, samoyed, northern inuit, English springer spaniel, boxer and a border collie. There were four intact females, six neutered females, six neutered males and four intact males. The average age was 7.45 ± 4.14 years (range 0.6 – 14.3 years). The average weight was 26.3 ± 9.3 kg (range 9.1 – 44.5 kg) (supplemental Table A, data available in Supplemental Material online). Table 1 displays selected descriptive statistics for LAV_{max} and LAV_{min} according to the method used to calculate LAV. Coefficients of variation for within day, between day and inter-observer variability of the four methods are listed in Table 2.

Real-time three-dimensional echocardiography and electrocardiographic-gated multidetector computed tomography angiography

Left atrial volume by RT-3DE was highly correlated with MDCTA for LAV_{max} (r = 0.94, p<0.0001) and LAV_{min} (r = 0.82, p<0.0001) (Fig. 4A and 4B respectively). However, on Bland-Altman analysis, RT-3DE slightly underestimated LAV_{max} by 6% compared to MDCTA (bias = -0.96 mL, 95% LoA: -5.6 – 3.7; Fig. 5A). Similarly, RT-3DE underestimated LAV_{min} by 6.7% (bias = -0.67 mL, 95% LoA: -5.40 – 4.06; Fig. 5B). Intraclass correlation (Table 3) confirmed these findings. There was almost perfect agreement between ECG-gated MDCTA and RT-3DE for LAV_{max} (ICC = 0.93, CI: 0.83 – 0.97) and LAV_{min} (ICC = 0.82, CI: 0.60 – 0.93).

Two-dimensional echocardiography and electrocardiographic-gated multidetector computed tomography angiography

Two-dimensional echocardiographic assessment of LAV also correlated well with MDCTA but not as highly as RT-3DE (LAV_{max} r = 0.81 {MOD} and 0.79 {ALM}, both p<0.0001, Fig. 4C and 4E; and LAV_{min} r = 0.70 {MOD} and 0.72 {ALM}, p<0.0006 and p<0.0004 respectively, Fig. 4D and 4F). However, Bland Altman analysis showed that MOD and ALM over-estimated LAV_{max} by 19.9% (bias = 3.18 mL, 95% LoA: -5.7 – 12.1) and 25.3% (bias = 4.05 mL, 95% LoA: -5.6 – 13.7) respectively (Fig. 5C and 5E). Minimum left atrial volume, was also overestimated, by 19.4% for MOD (bias = 1.96 mL, 95% LoA: -4.6 – 8.5) and 27.7% for ALM (bias = 2.80 mL, 95% LoA: -3.9 – 9.5) compared with MDCTA (Fig. 5D and 5F). The levels of agreement were less than that obtained by 3D imaging. Values obtained by 2D echocardiography also demonstrated substantial agreement with ECG-gated MDCTA for both LAV_{max} (ALM; ICC = 0.78, CI: 0.52 – 0.91; MOD; ICC = 0.71, CI: 0.40 – 0.87) and LAV_{min} (ALM; ICC = 0.80, CI: 0.56 – 0.92; MOD; ICC = 0.70, CI: 0.38 – 0.87).

Discussion

Real-time three-dimensional echocardiography is a new technique whose innovative use in people to quantify LAV has greatly expanded over the last decade. Multiple human studies have consistently shown that RT-3DE is a useful diagnostic tool that tends to underestimate LAV to a lesser extent than biplane ALM or MOD [12,13,24,27]. This is the first veterinary study to measure the degrees of accuracy and bias of a commercially available 3D echocardiography system, using semi-automated border detection to measure LAV in a population of canine patients.

We have demonstrated that RT-3DE in dogs is more accurate at measuring LAV than 2D echocardiography when compared with the gold standard of 64-slice ECG-gated MDCTA. Real-time three-dimensional echocardiography-derived values were in almost perfect agreement with those obtained by MDCTA, whereas 2D methods overestimated LAV with wider LoA. In agreement with the recent work of Tidholm et al. (2019), we conclude that 2D volume-based methods and RT-3DE cannot be used interchangeably to quantify LAV in dogs [37]. Although 2D measurements remain easier and quicker to perform, RT-3DE is feasible, more accurate, and highly reproducible, so that establishing canine reference ranges is a realistic goal.

We used an ECG-gated MDCTA volumetric gold standard because it is used to measure LAV in people with a high degree of accuracy, even when they have atrial fibrillation [24,25,38,39]. Furthermore, in dogs, ECG-gated MDCTA is already recognised as a volumetric gold standard for assessment of left and right ventricular volumes [40,41].

Accuracy of echocardiographic methods was determined in two stages. First, both RT-3DE and 2D echocardiography were shown to correlate highly with the volumetric gold standard. Second, Bland-Altman plots confirmed that RT-3DE outperformed 2DE with a minimal bias and the narrowest 95% LoA obtained for both LAV_{max} and LAV_{min}. The slight underestimation of RT-3DE that we recorded is consistent with reported biases in human medicine ranging from 0 to -2.5 mL [12,24].

Studies in people have consistently shown that 2D echocardiography, as well as RT-3DE, underestimates LAV compared to gold standard methods [12,13,15,20,24–27]. Surprisingly, in our study, we found that 2D planimetric methods overestimated LAV. The reason for this is unclear. It could be that differences in thoracic conformation and anatomical cardiac chamber anatomy mean that geometric assumptions that are valid in people do not apply to the canine LA [42]. Importantly, this discrepancy illustrates the pitfalls of extrapolating data across species when applying novel imaging modalities to veterinary medicine.

Two previous veterinary studies broadly agree with our data. They also demonstrated overestimation of LAV by both 2D planimetric methods, compared to RT-3DE [18] or cardiac magnetic resonance imaging [30], although cardiac magnetic resonance imaging does not appear to correlate with monoplane MOD, RT-3DE or even MDCTA. Furthermore, and similar to people [22,43,44], our MOD-derived values were closer to MDCTA values than ALM-derived ones, since biplane MOD makes fewer geometric assumptions than ALM [10]. This would suggest that in healthy dogs, although inferior to RT-3DE, biplane MOD remains the 2D planimetric method of choice for measuring LAV [10,37,43]. By contrast, in a recent Swedish study that included a large population of dogs with myxomatous mitral valve disease, biplane MOD underestimated LAV compared to RT-3DE [37]. Our data suggest that 2D geometric assumptions might be less accurate in dogs with bigger atria since there was a trend towards an increase in the scatter of the difference between MDCTA and planimetric measurements of LAV as atrial size increased.

Reduced accuracy at bigger LAV may relate to the views from which images were derived. Both RT-3DE and 2D images were obtained from left apical views. Apical imaging places LA in a far field of the ultrasound beam, leading to loss of lateral resolution, poor visualisation, and inaccurate tracing of the LA endocardial border [22,45]. Despite significant effort by the sonographer to obtain good quality non-foreshortened four- and two-chamber views by optimising the width and the depth, manual tracing errors may have contributed to LAV overestimation by including some of the LAA or pulmonary veins [46]. Indeed, unlike in people, canine pulmonary veins anastomose into a cone-shaped trunk prior to entering the LA. This leads to an imprecise delineation between left atrial tissue and the beginning of pulmonary veins [42]. By contrast, in RT-3DE, we used a semi-automated software package designed to provide an ellipsoidal 3D cast of the left ventricle after placing three references points on the endocardial border. Although this required manual adjustment, the degree of manual involvement was significantly less than with 2D techniques. It is possible that the software package is superior to manual selection of border delineation, resulting in greater inaccuracy where there is greater manual input i.e. 2D methods. Indeed, manual tracing of the endocardium is operator-dependant and tends to be associated with higher interobserver variability than RT-3DE [20]. Despite the reproducibility among the echocardiographic methods in our study being <15%, and hence clinically acceptable compared to previously reported human [12,20,24] and veterinary [18,43,47] studies, the effect size would be greater with bigger left atria, which have longer perimeters. We conclude that despite the advantages of RT-3DE over 2D imaging, RT-3DE requires separate validation for measuring LAV in disease states, using a large population of dogs.

These findings are clinically significant, since thoracic conformation can vary greatly in dogs of different morphotypes potentially leading to anatomical variation, including LAV [48,49]. Indeed, Hollmer et al. (2013) have documented significant breed-related differences in LAV using the biplane ALM in dogs with normal cardiovascular status [47]. By contrast, we demonstrated that RT-3DE maintained its bias over a range of LAV within brachycephalic, dolicocephalic and mesaticephalic breeds with slight underestimation. We speculate that RT-3DE would also demonstrate the same bias and degree of accuracy within breeds over a range of pathological LA remodelling. Additional research validating the use of RT-3DE measurements in dogs with enlarged left atria is warranted to confirm this hypothesis.

Our study has several noteworthy limitations that should be considered. It was not designed to correct for potential confounders such as heart rate, changes in LA loading conditions, sex, image quality and eventually variability throughout the breathing cycle, and no attempts were made to correlate these parameters with LAV. Sporadically, we found that sinus arrhythmia or heart rates greater than 80 beats per minute caused stair-step and motion artefacts on MDCTA. This was due to the limited temporal resolution of 64-slice ECG-gated MDCTA and the absence of breath holding during data acquisition. The consequence was poorer definition of the LA endocardial border, although we believe that this was not sufficient to lead to erroneous measurements because each patient served as his own control with short time-frame between modalities and therefore data acquisitions.

The confounding effects on respirophasic changes are likely to have been similar between the different methods used to measure LAV [50]. Lung expansion during end-inspiration decreases LAV, and conversely, LAV increases during end-expiration [50,51]. In our study, anaesthetised dogs were assisted by mechanical ventilation during both MDCTA and echocardiography. Images acquisition were not synchronised with the breathing cycle, which could theoretically influenced the loading condition of the LA. Synchronisation with the breathing cycle would have imply using breath-holding which was not undertaken not only for ethical reasons but also to mirror clinical situations. Besides, breath-holding could have influenced the loading of the LA by up to 20% compared to normal breathing [52].

Real-time three-dimensional echocardiography is also subject to limited temporal and spatial resolution during real-time acquisition. In previous veterinary studies, the optimal balance between spatial and temporal resolution was obtained by acquiring volume datasets with multi-beat modalities involving acquisition of four to seven cardiac cycles [16–18,53]. However, to avoid stitching artefact, this mode requires a regular heart rate (ideally between 60 and 90 beats per minute based on the author experience), breath-holding and stable probe handling from the operator. This is not easily achieved in a clinical veterinary setting. Our aim was to demonstrate the clinical feasibility of RT-3DE, and because inhalation anaesthetics can increase heart rate by decreasing cardiac vagal activity [54], single-beat acquisition was preferred at the expense of image quality. Overall image quality was deemed adequate for LAV estimation and our conclusions should be equally applicable to emerging 3D technologies with improved image quality.

It is also worth emphasising that frame selection varied due to image quality and frame rate. Consequently, measurements were not performed at exactly the same time point, potentially leading to underestimation of LA. However, we attempted to overcome this limitation by relying on the average of three different measurements for each variable for both MDCTA and RT-3DE.

The aim of this study was not to generate allometric relationships in order to establish reference values for RT-3DE and planimetric methods. Additional studies involving the use of RT-3DE in a larger population of dogs are required to establish reference values in healthy and conscious patients, and to optimise the clinical utility of RT-3DE to estimate LAV.

Conclusions

This is the first prospective study in veterinary medicine to study the accuracy and the bias of RT-3DE with a volumetric gold standard to estimate LAV. Three-dimensional-derived LAV measurements were found to be feasible, reproducible and more accurate than 2D echocardiography-based volumetric methods. Our results might facilitate future clinical research in order to determine a general consensus for the best method to assess LAV. Although RT-3DE is not currently widely available in veterinary medicine, this modality remains appealing and holds promise for LAV assessment. It is anticipated that this imaging modality will emerge as a powerful diagnostic tool and will be integrated into clinical guidelines and routine practice as the imaging modality of choice for LA assessment with dedicated software and adapted algorithms alongside the improvement of spatial and temporal resolution in the next generation of 3D probes.

Conflicts of Interest Statement

The authors do not have any conflicts of interest to disclose

Footnotes

a. Cardell Model 9402, CAS Medical Systems, Brandford, Connecticut.
b. Acecare, Animalcare limited, United Kingdom
c. Alvegesic, Dechra Veterinary products, United Kingdom
d. Comfortan, Dechra Veterinary products, United Kingdom
e. Dexmedetomidine, Orion Pharma, Turku, Finland
f. Propoflo plus, Zoetis UK Ltd, United Kingdom
g. Isocare, Animalcare Ltd, United Kingdom
h. Vivid E9, General Electric Medical Systems, Waukesha, Wisconsin.
i. 4V-D Active Matrix 4D Volume Phased Array Transducer 1.5 – 4.0MHz
j. M5S-D Active Matrix single-crystal Phased Array Transducer 1.5 – 4.6MHz
k. EchoPAC PC versions 113, GE Healthcare, Horten, Norway
l. Somatom Sensation 64, Siemens Medical Solutions, Erlangen, Germany.
m. Care Dose 4D®, Siemens Healthcare, Forchheim, Germany
n. Niopam 350®, Bracco UK Ltd, United Kingdom
o. Empower CTA®+, Bracco® injeneering S.A., Milan, Italy
p. 4D-AutoLVQ, GE Healthcare, Horten, Norway
q. OsiriX v5.8.5 64-bit, Geneva, Switzerland
r. GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla, California, USA.
s. Minitab 17, Pennsylvania, USA
t. MedClac Stasticial Software, version 18.5, MedCalc Software bvba, Ostend, Belgium

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Fig. 1

Representative real-time biplane echocardiography images of the left atrium just before mitral valve opening corresponding to maximum left atrial volume (A and B) and left atrium just after mitral valve closure corresponding to minimum left atrial volume (C and D) were obtained. The endocardial borders were traced manually while excluding the left auricular appendage (*) and the ostia of pulmonary veins, if visible. The mitral annulus was considered as the left atrioventricular border. The cut plane was adjusted from the left parasternal four-chamber view (A and C) in order to obtain a non-foreshortened two-chamber view (B and D).

A: Left parasternal four-chamber view (0°) when left atrium is maximum. B: Left parasternal two-chamber view (90°) when left atrium is maximum. C: Left parasternal four-chamber view (0°) when left atrium is minimum. D: Left parasternal two-chamber view (90°) when left atrium is minimum.

LA: left atrium; LV: left ventricle; RA: right atrium.

Fig. 2

Representative real-time three-dimensional echocardiography images of the left atrium in a dog. After automatic slicing of real-time three-dimensional echocardiography data set and manual alignment, manual input for placing reference points was carried out (one point at the dorsal border of the left atrium, one point at the hinge-point of the septal leaflet and one point at the hinge-point of the lateral leaflet). Following this step, automatic detection of endocardial surface in three dimensions was generated. In this example, manual adjustment by adding a total of 3 points in left apical four-chamber view (A), 3 points in left apical two-chamber view (B) and 5 points in left apical five-chamber view (C) was performed. These manual adjustments permitted accurate delineation of the left atrium cavity prior volume measurement by the software. Left atrial cast associated to the time-volume curve (E) were obtained. Notice the electrocardiographic tracing at the lower edge of the image correspond to the image just before mitral valve opening corresponding therefore to maximum left atrial volume. A: left apical four-chamber view. B: left apical two-chamber view. C: left apical five-chamber view. D: short axis view of the left atrium. Note that the plane can be adjusted through a perpendicular axis from the roof of left atrium to the mitral valve annulus. E: automatically reconstructed three-dimensional left atrial cavity cast and its corresponding typical time-volume curve (y axis indicates left atrial volume {mL}; x-axis indicates time {s}). ED: end atrial diastole corresponding to maximum left atrial volume; ES: end atrial systole corresponding to minimum left atrial volume

Fig. 3

Cardiac electrocardiographic-gated multi-detector computed tomography angiography in sagittal (A) and axial (E) planes. Regions of interest delineate the left atrium and extend from the dorsal border of the left atrium to the mitral valve annulus (B, C, D, F, G, H, J, K, L). Image I represents the computed three-dimensional volume.

Ao: aorta; LA: left atrium; *: left auricle; LV: left ventricle; RV: right ventricle.

Fig. 4

Correlations between real-time three-dimensional echocardiography (RT-3DE), biplane method of disk (MOD) and biplane area-length method (ALM) with the reference method multi-detector computed tomography angiography (MDCTA) for maximum left atrial volume (top) and minimum left atrial volume (bottom) of 20 anesthetised dogs.

When left atrial volume is maximal, there were a positive correlations between MDCTA and RT-3DE (r = 0.93; p<0.0001) (A), MDCTA and biplane MOD (r = 0.81; p<0.0001) (C), and MDCTA and biplane ALM (r = 0.79; p<0.0001) (E).

Similarly, when left atrial volume is minimal, there were a positive correlations was detected when comparing MDCTA and RT-3DE ($r = 0.82$; $p<0.0001$) (B), MDCTA and biplane MOD ($r = 0.7$; $p = 0.0006$) (D), and MDCTA and biplane ALM ($r = 0.72$; $p = 0.0004$) (F).

Solid lines represent the coefficient of correlation; dashed lines represent the 95% confidence intervals.

Note that all methods correlate better for maximum left atrial volume.

ALM: area-length method; LAV_{max}: maximal left atrial volume; LAV_{min}: minimal left atrial volume; MDCTA: multi-detector computed tomography angiography; MOD: method of disk; RT-3DE: real-time three-dimensional echocardiography.

Fig. 5

Bland-Altman analysis showing the agreement between maximal (top) and minimal (bottom) left atrial volumes measured by the reference method, multi-detector computed tomography angiography, and those measured by real-time three-dimensional echocardiography (RT-3DE) (A and B), biplane method of disk (MOD) (C and D) or biplane area-length method (ALM) (E and F). Solid lines represent the mean difference (bias) and dashed lines represent the 95% limits of agreement (± 2 SD from the mean between the two techniques used).

Note that MOD and ALM consistently overestimated left atrial volume, particularly at larger volume. By contrast, RT-3DE underestimated slightly left atrial volumes.

ALM: area-length method; MDCTA: multi-detector computed tomography angiography; MOD: method of disk; RT-3DE: real-time three-dimensional echocardiography; SD: standard deviation.

Table A (supplementary data): Epidemiological and clinical characteristics of the 20 dogs included in the study.

FE: female entire; FS: female spayed; MDCTA: multi-detector computed tomography angiography; ME: male entire; n.a: not available; NM: neutered male

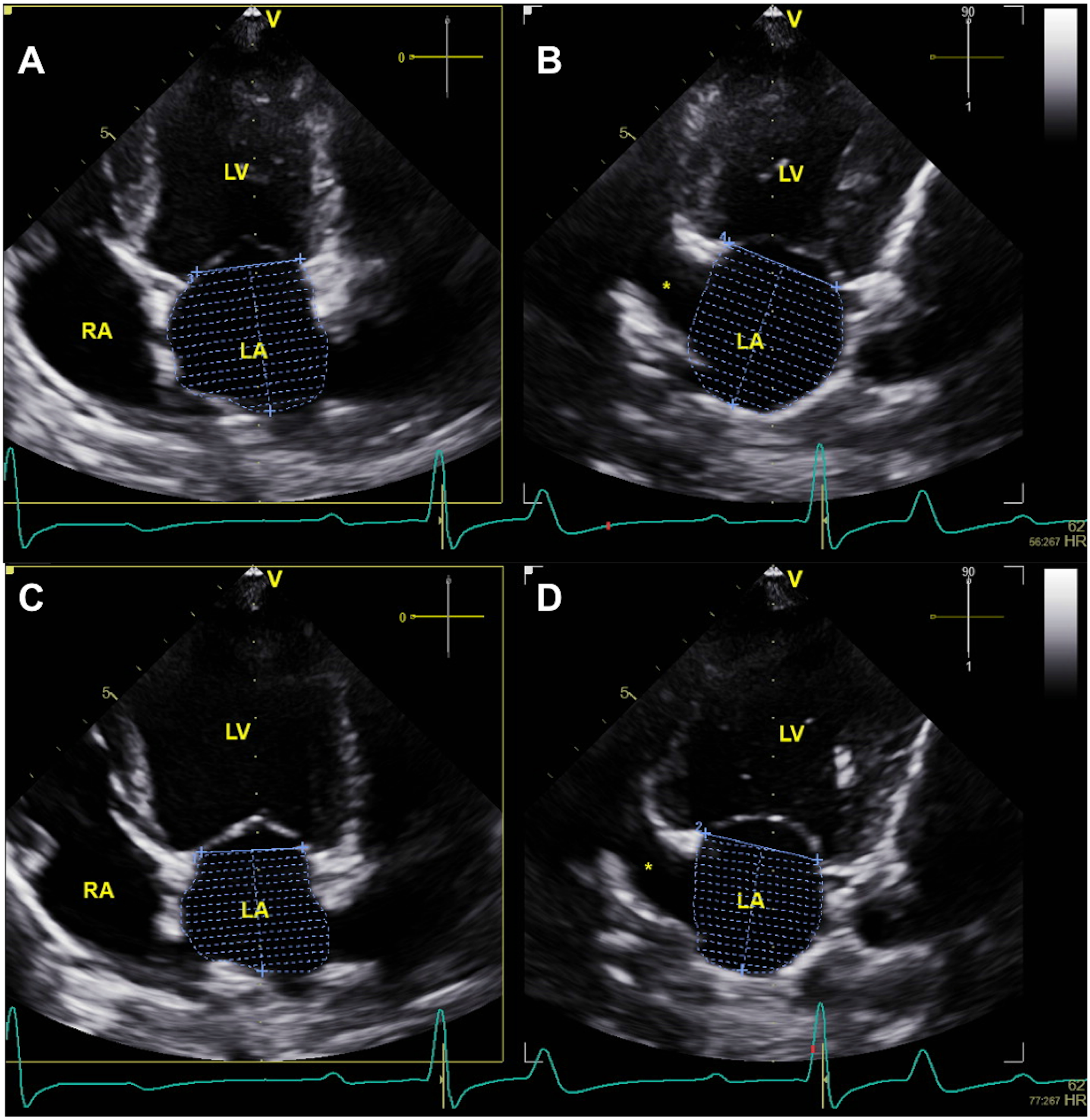
Table 1: Descriptive statistics of left atrial volume during maximum and minimum left atrial size. ALM: area-length method; LAV_{max}: maximal left atrial volume; LAV_{min}: minimal left atrial volume; MDCTA: multi-detector computed tomography angiography; MOD: method of disk; RT-3DE: real-time three-dimensional echocardiography.

Table 2: Coefficient of variation for measurement of minimum left atrial volume and maximum left atrial volume according to different methods.

ALM: area-length method; CV: coefficient of variation; LAV_{max}: maximal left atrial volume; LAV_{min}: minimal left atrial volume; MDCTA: multi-detector computed tomography angiography; MOD: method of disk; RT-3DE: real-time three-dimensional echocardiography.

Table 3: Intraclass correlation coefficients and 95% CI reported for each part of measurements and for each parameter measured. Coefficient interpretation: 0 – 0.2 indicates poor agreement; 0.3 – 0.4 indicates fair agreement; 0.5 – 0.7 indicates moderate agreement; 0.7 – 0.8 indicates strong agreement; and > 0.8 indicates almost perfect agreement.

ALM: area-length method; CI: confidence interval; ICC: intraclass correlation; LAV_{max}: maximal left atrial volume; LAV_{min}: minimal left atrial volume; MDCTA: multi-detector computed tomography angiography; MOD: method of disk; RT-3DE: real-time three-dimensional echocardiography.



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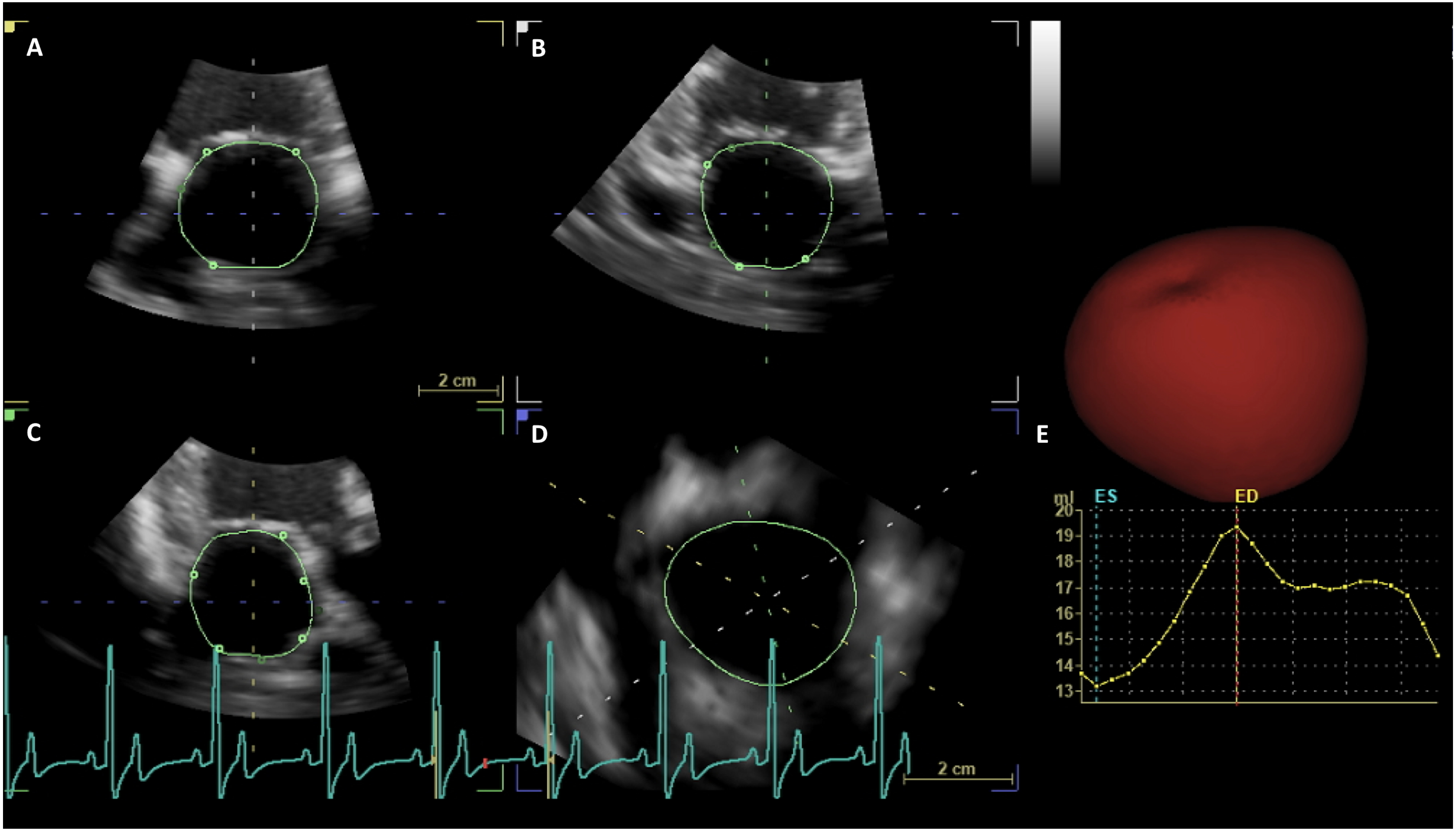

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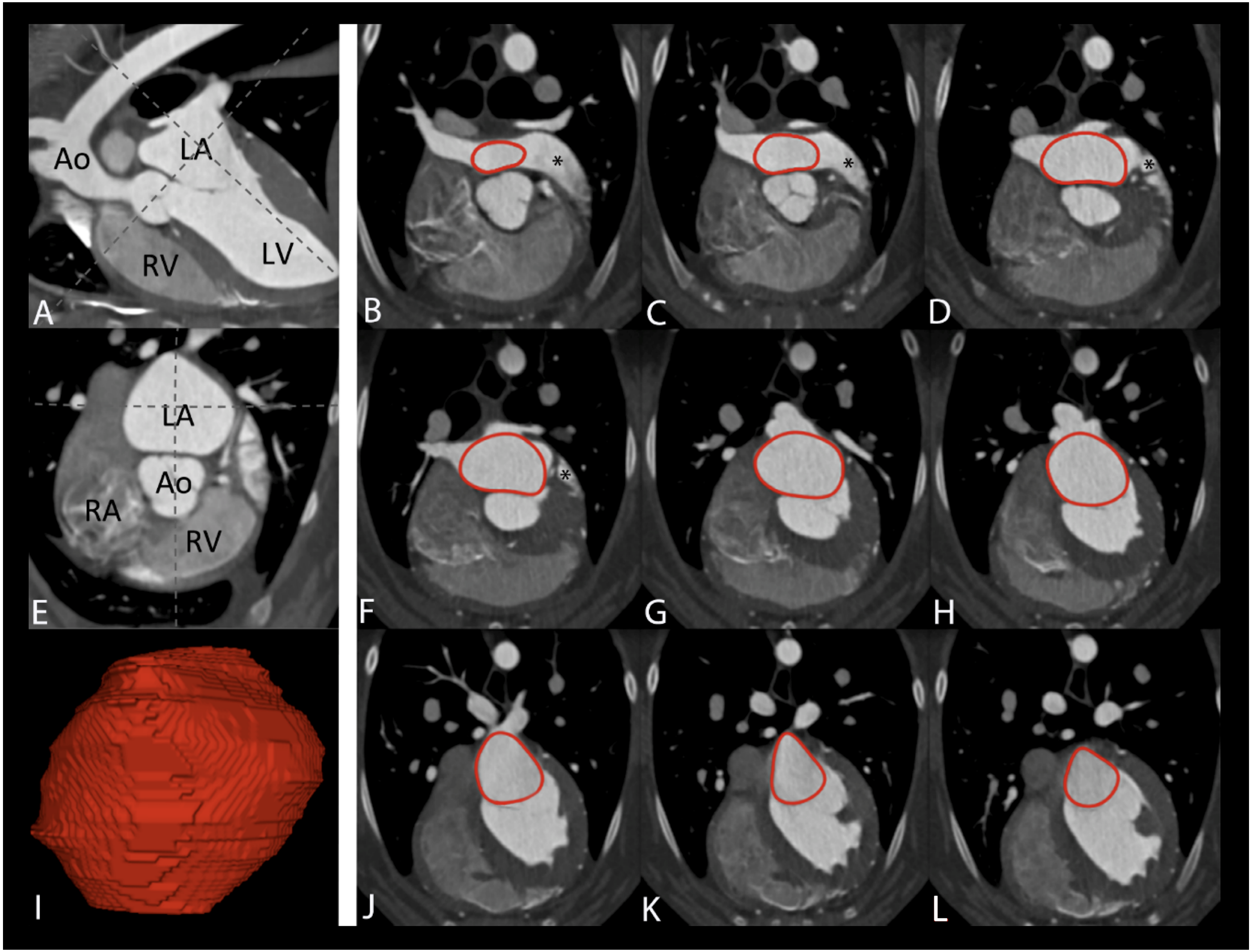

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LV
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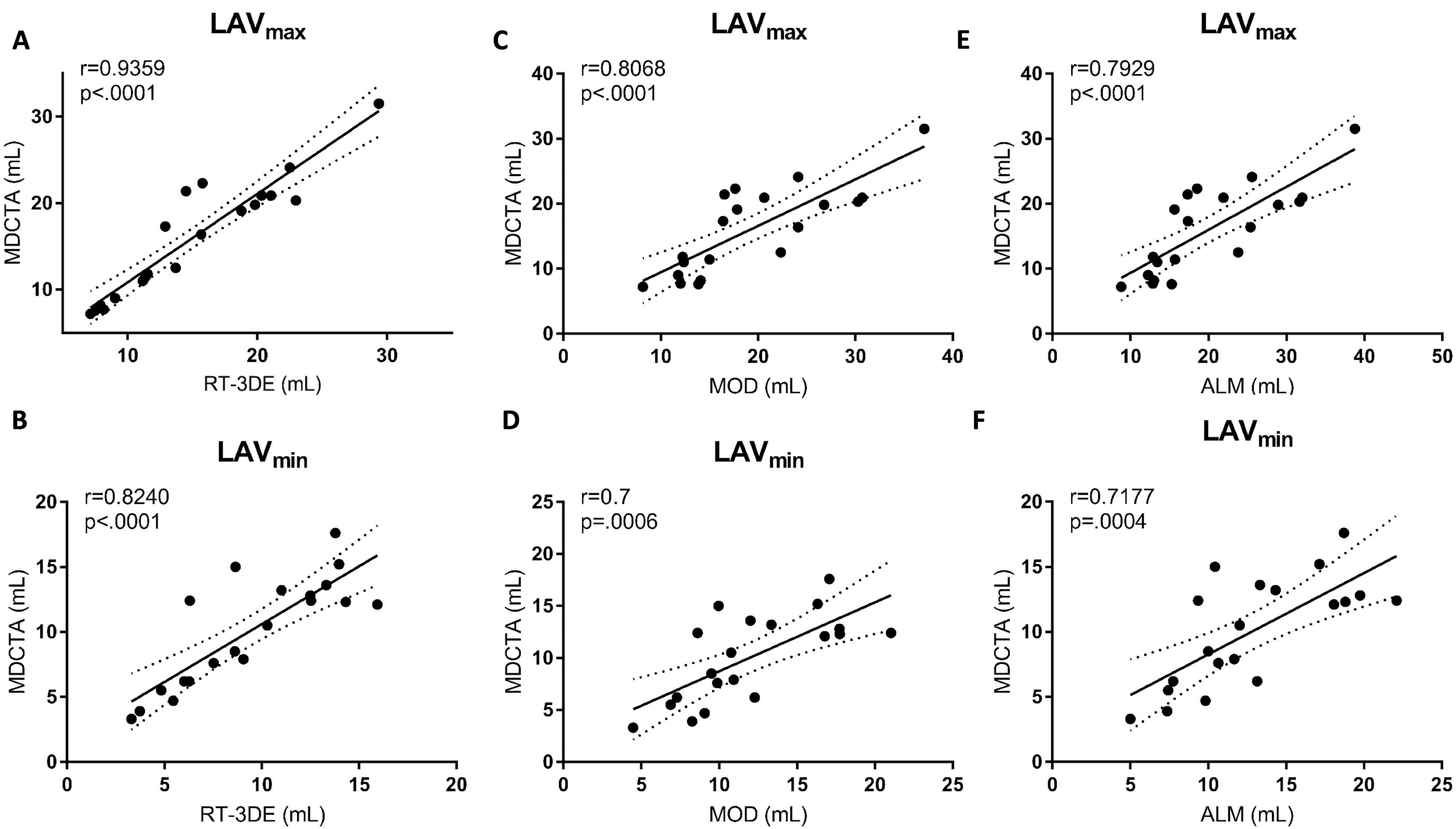
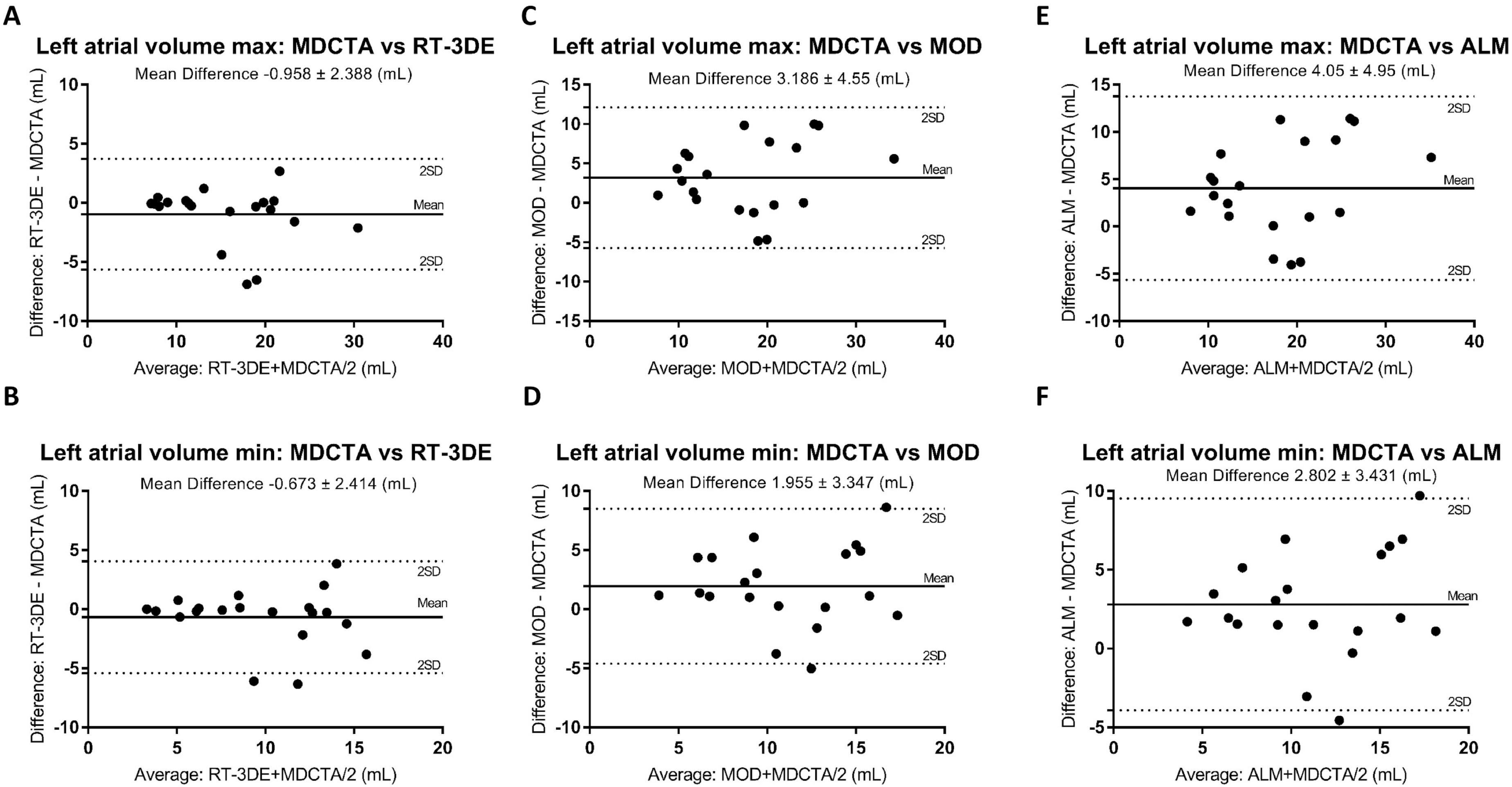
B
C
D
*
*
*
LA
Ao
RA
RV
E
F
G
H
*
I
J
K
L



A
LAVmax
r=0.9359
p<.0001
MDCTA (mL)
RT-3DE (mL)
C
LAVmax
r=0.8068
p<.0001
MDCTA (mL)
MOD (mL)
E
LAVmax
r=0.7929
p<.0001
MDCTA (mL)
ALM (mL)
B
LAVmin
r=0.8240
p<.0001
MDCTA (mL)
RT-3DE (mL)
D
LAVmin
r=0.7
p=.0006
MDCTA (mL)
MOD (mL)
F
LAVmin
r=0.7177
p=.0004
MDCTA (mL)
ALM (mL)


A
Left atrial volume max: MDCTA vs RT-3DE
Mean Difference -0.958 ± 2.388 (mL)
Difference: RT-3DE - MDCTA (mL)
Average: RT-3DE+MDCTA/2 (mL)
2SD
Mean
2SD
C
Left atrial volume max: MDCTA vs MOD
Mean Difference 3.186 ± 4.55 (mL)
Difference: MOD - MDCTA (mL)
Average: MOD+MDCTA/2 (mL)
E
Left atrial volume max: MDCTA vs ALM
Mean Difference 4.05 ± 4.95 (mL)
Difference: ALM - MDCTA (mL)
Average: ALM+MDCTA/2 (mL)
B
Left atrial volume min: MDCTA vs RT-3DE
Mean Difference -0.673 ± 2.414 (mL)
Difference: RT-3DE - MDCTA (mL)
Average: RT-3DE+MDCTA/2 (mL)
D
Left atrial volume min: MDCTA vs MOD
Mean Difference 1.955 ± 3.347 (mL)
Difference: MOD - MDCTA (mL)
Average: MOD+MDCTA/2 (mL)
F
Left atrial volume min: MDCTA vs ALM
Mean Difference 2.802 ± 3.431 (mL)
Difference: ALM - MDCTA (mL)
Average: ALM+MDCTA/2 (mL)

† Evans E. H, De Lahunta A. The Skeleton. In: Evans E. H, De Lahunta A, editors. Miller's Anatomy of the Dog. 4th ed. St. Louis, MO: Saunders; 2013, p. 86–7.

‡ Schwarz T, Sullivan M, Hartung K. Radiographic anatomy of the cribiform plate (lamina cribrosa). Vet Radiol Ultrasound 2000;41:220–5.

Table A (supplementary data): Epidemiological and clinical characteristics of the 20 dogs included in the study.

FE: female entire; FS: female spayed; MDCTA: multi-detector computed tomography angiography; ME: male entire; n.a: not available; NM: neutered male

Dog	Breed	Sex	Age (days)	Body weight (kg)	Reason for MDCTA	Final diagnosis	Morphotype [†,‡]
1	Cross breed	FS	1780	13	Cough	Chronic lower airway inflammation	mesaticephalic
2	Labrador Retriever	ME	268	28	Cough	Normal airway	mesaticephalic
3	Flat Coated Retriever	FE	2500	23.7	Nasal discharge	Aspergillosis	Mesati-Dolichocephalic
4	Cross breed	NM	1956	29.6	Nasal discharge	Aspergillosis	mesaticephalic
5	Golden Retriever	FS	3123	35.8	Cough	Bordetella Bronchiseptica	mesaticephalic
6	Schnauzer	ME	3617	14	Staging	Prostatic adenocarcinoma	n.a
7	Collie Rough	FS	4029	28.9	Staging	Mandible osteosarcoma	mesaticephalic
8	Golden Retriever	FE	4542	33.3	Exercise intolerance	Normal airway	n.a
9	Labrador Retriever	FS	3993	27.5	Staging	Oral malignant melanoma	Brachy-Mesaticephalic
10	Samoyed	FR	3493	27	Staging	Nasal transitional cell carcinoma	Brachy-Mesaticephalic
11	Cocker Spaniel	FS	747	10.7	Cervical mass	Neutrophilic pyogranulomatous inflammation	Mesati-Dolichocephalic

12	Golden Retriever	FR	222	18.85	exophthalmos	Retrobulbar abscess	mesaticephalic
13	Cross breed	ME	410	9.1	Cough	Tracheal collapse	mesaticephalic
14	Cross breed	NM	5218	25	Staging	Oral squamous cell carcinoma	mesaticephalic
15	Northern Inuit	NM	2324	44.5	Staging	Lymphoma	mesaticephalic
16	English Springer Spaniel	FS	3007	25.5	Sneezing	Nasal foreign body	mesaticephalic
17	Cocker Spaniel	NM	2502	14	Cough	Pulmonary foreign body	mesaticephalic
18	Border Collie	ME	2034	22.3	Nasal discharge	Rhinitis	mesaticephalic
19	Labrador Retriever	NM	4118	36	Cough	Bronchopneumonia	n.a
20	Boxer	NM	4542	30.3	Staging	Soft tissue sarcoma	brachycephalic

Table 1: Descriptive statistics of left atrial volume during maximum and minimum left atrial size. ALM: area-length method; LAV_{max}: maximal left atrial volume; LAV_{min}: minimal left atrial volume; MDCTA: multi-detector computed tomography angiography; MOD: method of disk; RT-3DE: real-time three-dimensional echocardiography.

Variable	Mean	Standard deviation	Minimum	Maximum
LAV_{max} (mL)				
Biplane ALM	20.1	8.19	8.8	38.8
Biplane MOD	19.2	7.6	8.2	37.1
RT-3DE	15.1	6.2	7.1	29.4
MDCTA	16.0	6.7	7.2	31.5
LAV_{min} (mL)				
Biplane ALM	12.9	4.8	5.0	22.1
Biplane MOD	12.0	4.4	4.5	21.0
RT-3DE	9.4	3.8	3.3	15.9
MDCTA	10.1	4.21	3.3	17.6
LAV_{max} (mL/kg)				
Biplane ALM	0.63	0.31	0.29	1.43
Biplane MOD	0.68	0.34	0.32	1.55
RT-3DE	0.63	0.17	0.38	0.89
MDCTA	0.39	0.20	0.18	0.90
LAV_{min} (mL/kg)				
Biplane ALM	0.35	0.18	0.17	0.81
Biplane MOD	0.40	0.20	0.19	0.91
RT-3DE	0.39	0.11	0.25	0.56
MDCTA	0.19	0.09	0.09	0.43

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Table 2: Coefficient of variation for measurement of minimum left atrial volume and maximum left atrial volume according to different methods.

ALM: area-length method; CV: coefficient of variation; LAV_{max}: maximal left atrial volume; LAV_{min}: minimal left atrial volume; MDCTA: multi-detector computed tomography angiography; MOD: method of disk; RT-3DE: real-time three-dimensional echocardiography.

	LAV_{min} RT-3DE	LAV_{max} RT-3DE	LAV_{min} MOD	LAV_{max} MOD	LAV_{min} ALM	LAV_{max} ALM	LAV_{min} MDCTA	LAV_{max} MDCTA
CV interobserver (%)	6.45	4.82	14.8	5.73	13.87	7.67	4.86	7.27
CV within day (%)	1.48	1.67	3.41	3.99	3.59	4.43	2.97	3.51
CV between day (%)	2.31	1.69	4.10	3.85	3.99	4.19	5.70	2.74

Table 3: Intraclass correlation coefficients and 95% CI reported for each part of measurements and for each parameter measured. Coefficient interpretation: 0 – 0.2 indicates poor agreement; 0.3 – 0.4 indicates fair agreement; 0.5 – 0.7 indicates moderate agreement; 0.7 – 0.8 indicates strong agreement; and > 0.8 indicates almost perfect agreement.

ALM: area-length method; CI: confidence interval; ICC: intraclass correlation; LAV_{max}: maximal left atrial volume; LAV_{min}: minimal left atrial volume; MDCTA: multi-detector computed tomography angiography; MOD: method of disk; RT-3DE: real-time three-dimensional echocardiography.

	LAV_{max} MDCTA			LAV_{min} MDCTA	
	ICC	95% CI		ICC	95% CI
LAV_{max} RT-3DE	0.9322	0.8371 to 0.9726	LAV_{min} RT-3DE	0.8215	0.6032 to 0.9253
LAV_{max} ALM	0.7801	0.5244 to 0.9068	LAV_{min} ALM	0.7109	0.4017 to 0.8746
LAV_{max} MOD	0.8010	0.5635 to 0.9162	LAV_{min} MOD	0.6989	0.3815 to 0.8689

Journal of Veterinary Cardiology

The following information is required for submission:

Author contribution

The ICMJE recommends that authorship be based on the following 4 criteria:

1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
2. Drafting the work or revising it critically for important intellectual content; AND
3. Final approval of the version to be published; AND
4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Please specify the contribution of **each author** to the paper, e.g. study concept or design, data collection, data analysis or interpretation, writing the paper, others, who have contributed in other ways, should be listed as contributors.

Yolanda Martinez Pereira, Florence Thierry, Geoff Culshaw, Tobias Schwarz and Ian Handel contributed substantially to the conception of the work and revising of the work and finally approving of the work and agree to accountable for all aspects of the work

As **Corresponding Author** I hereby confirm that all listed authors in the submission meet these Criteria.

Corresponding author: Jonathan Bouvard

Please add signature here:

Date: 09/11/2018