Transthoracic two-dimensional and three-dimensional echocardiography for the measurement of mitral valve area planimetry in English Bull Terriers with and without heart disease


Published in:
Journal of Veterinary Cardiology

DOI:
10.1016/j.jvc.2021.06.002

Publication date:
2021

Document version:
Accepted manuscript

Document license:
CC BY-NC-ND

Citation for published version (APA):

Go to publication entry in University of Southern Denmark's Research Portal

Terms of use
This work is brought to you by the University of Southern Denmark. Unless otherwise specified it has been shared according to the terms for self-archiving. If no other license is stated, these terms apply:

- You may download this work for personal use only.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying this open access version

If you believe that this document breaches copyright please contact us providing details and we will investigate your claim. Please direct all enquiries to puresupport@bib.sdu.dk

Download date: 15. Sep. 2023
Transthoracic Two-Dimensional and Three-Dimensional Echocardiography for the Measurement of Mitral Valve Area Planimetry in English Bull Terriers with and without Heart Disease

Chayanon Chompoosan, MSc, Anders Simon Schrøder, DVM, Maiken Bayer Thode Bach, DVM, Rasmus Møgelvang, PhD, Jakob Lundgren Willesen, PhD, Rebecca Langhorn, PhD, Jørgen Koch, PhD

PII: S1760-2734(21)00078-3
DOI: https://doi.org/10.1016/j.jvc.2021.06.002
Reference: JVC 714

To appear in: Journal of Veterinary Cardiology

Received Date: 2 June 2020
Revised Date: 7 June 2021
Accepted Date: 10 June 2021


This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2021 Elsevier B.V. All rights reserved.
Transthoracic Two-Dimensional and Three-Dimensional Echocardiography for the Measurement of Mitral Valve Area Planimetry in English Bull Terriers with and without Heart Disease

Chayanon Chompoosan\textsuperscript{a}, MSc., Anders Simon Schrøder\textsuperscript{a}, DVM., Maiken Bayer
Thode Bach\textsuperscript{a}, DVM., Rasmus Møgelvang\textsuperscript{b}, PhD, Jakob Lundgren Willesen\textsuperscript{a}, PhD,
Rebecca Langhorn\textsuperscript{a}, PhD, Jørgen Koch\textsuperscript{a}, PhD

\textsuperscript{a}Department of Veterinary Clinical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark
\textsuperscript{b}Department of Clinical Research, University of Southern Denmark, Svendborg, Denmark

Corresponding author: Jørgen Koch, koch@sund.ku.dk

Running head: THREE-DIMENSIONAL ECHOCARDIOGRAPHY IN ENGLISH BULL TERRIERS

Acknowledgments
This study was financially supported by the Department of Veterinary Clinical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark; by the Faculty of Veterinary Sciences, Mahasarakham University, Maha sarakham, Thailand, and by the English Bull Terrier club in Denmark. The authors would like to thank Marianne Duchwaider, veterinary technicians Elinor Aili Rikovitz Jørgensen & Tenna Bandsberg Pedersen for their participation in the data collection.
Abstract

Introduction: Mitral valve area (MVA) planimetry is used to diagnose and classify mitral stenosis (MS) in humans using two- and three-dimensional echocardiography (MVA$_{2D}$ and MVA$_{3D}$). This study aimed to evaluate agreement, feasibility and observer variability between MVA$_{2D}$ and MVA$_{3D}$ in English Bull Terriers (BT). Our hypotheses were 1) that the MVA of BT is generally smaller than that of breeds with similar body weight and 2) that these techniques could be used to diagnose MS in BT.

Animals: Twenty healthy BT, 15 healthy Boxers, and 49 BT with heart disease.

Materials and Methods: A prospective diagnostic agreement study was conducted. All dogs underwent a thorough clinical examination, conventional transthoracic echocardiography, and three-dimensional echocardiography.

Results: Bland-Altman plots (limits of agreement: 0.12–1.5) showed consistent bias and poor agreement between MVA$_{2D}$ and MVA$_{3D}$. For the 69 BT, MVA$_{3D}$ (2.1 ± 0.5 cm$^2$) measurements were significantly lower than MVA$_{2D}$ measurements (2.9 ± 0.6 cm$^2$), and healthy BT had significantly lower MVA parameters than healthy Boxers (p <0.001). Intra- and inter-observer variability were excellent for both MVA$_{2D}$ and MVA$_{3D}$ (intraclass correlation coefficient >0.9). Six BT were diagnosed with MS, with MVA$_{3D}$ less than 1.8 cm$^2$ and a mean transmitral gradient (MTG) of more than 5 mmHg.

Conclusions: Both MVA$_{2D}$ and MVA$_{3D}$ are feasible, have low observer variability and can be used to diagnose MS in BT. To assess the narrowest orifice area, the preferred method is MVA$_{3D}$. The smaller MVA in BT compared to Boxers may indicate some degree of MS.
KEYWORDS

Boxer; Canine; Mitral stenosis; Mitral valve orifice.
Abbreviations

BT        english bull terrier
bpm       beats per minute
BSA       body surface area
MS        mitral stenosis
MTG       mean transimtrial gradient
MVA       mitral valve area
MVA\textsubscript{2D} mitral valve area measurement by planimetry using two-dimensional echocardiography
MVA\textsubscript{3D} mitral valve area measurement by planimetry using three-dimensional echocardiography
MVD       mitral valve disease
Introduction

The English Bull Terrier (BT) is highly predisposed to heart diseases with a complex cardiac phenotype, including mitral valve disease (MVD) [1,2] with or without mitral stenosis (MS) [3,4], aortic stenosis (AS), myocarditis and extensive early-onset arteriosclerosis [1]. The majority of these dogs have an early-onset of disease and are below 3 years of age at the time of diagnosis [1]. Transthoracic echocardiography is considered the first-line diagnostic tool for diagnosis of MS in dogs. Currently, there is no established gold standard or cut-off values for diagnosing MS in dogs – except for preliminary normal reference ranges for mitral valve area (MVA) established in only 17 dogs of various size and breeds [5].

A case series from Lehmkuhl et al. (1994) defined the criteria for diagnosing MS including mitral valve motion characteristic for valve stenosis, increased mitral valve inflow velocity and a prolonged pressure half-time [3]. However, use of echocardiographic parameters other than MVA to diagnose MS increases the risk of over-interpreting functional MS as an anatomical MS [6]. In the literature, only one case report has been published where MVA measurements by planimetry using two-dimensional echocardiography (MVA$_{2D}$) was measured in a BT with MS [7].

According to the guidelines in human medicine, several methods of echocardiography can be used to assess the severity of MS including MVA parameters, mitral inflow parameters, systolic pulmonary artery pressure and valve anatomy. In routine evaluation, the MVA can be measured by planimetry and the pressure half-time methods. Furthermore, MVA and a mean transmitral gradient (MTG) should be used together for the assessment of the severity of MS. If there is a discrepancy between these methods, MVA planimetry is regarded as the reference method [8].
Obtaining MVA measurements by planimetry using two-dimensional or three-dimensional echocardiography (MVA_{3D}) is advantageous compared to indirect methods such as Doppler calculation from the pressure half-time due to the effects from left ventricular compliance [9,10], atrial fibrillation [11], mitral regurgitation [11-13], aortic regurgitation [10,12], and concomitant valvular diseases including aortic valve disease [9] and combined MS and mitral regurgitation [13] influencing the Doppler measurements.

However, there are some limitations to MVA_{2D} as it requires the parasternal window and an experienced echocardiographer [14]. If the short-axis image does not cross the mitral valve tips, MVA_{2D} may be overestimated [15]. Furthermore, this technique seems less reliable in dogs due to poor resolution and improper selection of the frame for measurement and gain setting [15]. In contrast, MVA_{3D} can be measured from both the parasternal and apical windows with no geometric assumption [14]. Several studies in humans indicate that MVA_{3D} is a more feasible, accurate, and reproducible method than MVA_{2D} [16-18].

This study aimed to compare MVA_{2D} and MVA_{3D} planimetry for MVA measurement and to evaluate the agreement, feasibility and observer variability of both in BT with and without heart disease. Furthermore, this study aimed to determine whether the mitral valve area (MVA_{2D} and MVA_{3D}) of healthy BT differs from the MVA of healthy Boxers, a breed with similar body surface area (BSA). Finally, we aimed to investigate the use of MVA_{2D} and MVA_{3D} for diagnosing MS in BT.

**Animals, Materials and Methods**

A prospective diagnostic agreement study was conducted at the University Hospital for Companion Animals, University of Copenhagen during the period 2014–2020. The inclusion criteria required that all dogs were more than 1 year of age at the time
of examination. A thorough clinical examination was carried out for each dog, including electrocardiography, blood pressure measurement, conventional transthoracic echocardiography and three-dimensional echocardiography. In addition, serum biochemistry was performed to obtain measurements of blood urea nitrogen and creatinine, and a urine protein/creatinine ratio was measured. The study was approved by the Ethical Committee (EAU 2017-13) at the Department of Veterinary Clinical Sciences, University of Copenhagen, Denmark, and written owner consent was obtained for all dogs.

**Echocardiography**

Echocardiographic studies were performed by a single experienced operator (JK) using an echocardiographic system equipped with a 6S MHz, M5Sc MHz, and a 4V MHz phased-array transducer. A conventional echocardiographic protocol was followed according to previously published guidelines [19,20]. This included transthoracic two-dimensional, M-mode, spectral and color-flow Doppler echocardiography with ECG recorded continuously. All dogs were examined without sedation and were gently manually restrained in right and left lateral recumbency.

Echocardiographic data were stored digitally for the offline assessment using an ultrasound software package.

The MVA measurement was obtained with a four-dimensional preparation. The four-dimensional zoom prepare mode was applied to the two-dimensional images of the left apical two- and four-chamber views, and the volume of interest included all of the mitral valve. A four-dimensional construction was then prepared. The offline analysis was initiated by selecting the frame that had a maximal mitral valve opening during early diastole. The three-dimensional Flexi Slice mode was applied to allow for image rotation, and multiplanar reconstruction was used to display the narrowest
cross-sectional opening of the mitral orifice. The edge of the mitral valve orifice was
then traced in a constructed two-dimensional image (Fig. 1).
The MVA_{2D} measurement was obtained in the right parasternal short-axis view (Fig.
2A). The apex to the base of the left ventricle was scanned to ensure that the image
crossed the mitral leaflet tips. The scan-plane position was parallel to the mitral valve
orifice. Measurements were obtained in early diastole when the valve was maximally
open. The gain setting was optimized to visualize the entire circumference of the
MVA, and the MVA was then traced [8]. The MVA was indexed to BSA [21,22], which
was calculated using the formula $0.10 \times \text{kg}^{0.667}$ [23].

All echocardiographic measurements were performed offline. The operators were
blinded to any information about the dogs. Dogs were defined as healthy when no
clinical abnormality was found based on physical examination, electrocardiography,
血 pressure measurement and when echocardiographic examination presented
normal cardiac structure and function without flow abnormalities.

Preliminary reference ranges for MVA_{2D} and MVA_{3D} were generated using the Boxer
- a dog breed with a similar BSA. The MVA_{2D} and MVA_{3D} preliminary reference
ranges were also generated in healthy BT and BT with heart disease to define the
diagnostic criteria for diagnosing MS.

Mitral valve disease was defined as the presence of mitral regurgitation, thickened
mitral leaflets or mitral valve prolapse [24]. A preliminary diagnosis of MS required
MVA_{3D} measurements less than the minimum value of the healthy BT group, MTG
more than 5 mmHg [3], thickened mitral leaflets, and mitral valve motion
characteristics of valve stenosis on the two-dimensional image including incomplete
diastolic leaflet separation and restricted diastolic movement. Aortic stenosis was
diagnosed in dogs with or without visible obstruction, if the peak left ventricular
outflow and aortic velocity from the subcostal transhepatic view was more than 2.4 m/s [25,26]. Pulmonic stenosis was diagnosed when peak pulmonic velocity was more than 1.8 m/s [25,27], with or without visible obstruction. Dilated cardiomyopathy was diagnosed based on previously reported guidelines [28], with the major criteria including increased left ventricular dimension/volume and decreased fractional shortening or left ventricular ejection fraction.

**Intra- and inter-observer variability**

A total of ten BT were randomly selected from the healthy BT and BT with heart diseases for the intra- and inter-observer variability. The MVA$_{2D}$ and MVA$_{3D}$ were measured three times on a triple-cycle echo loop/image and averaged in all ten dogs by two blinded observers (JK and CC) for inter-observer variability. One week later one blinded observer (JK) repeated the measurements (measuring the MVA$_{2D}$ and MVA$_{3D}$ in each dog three times on a triple-cycle echo loop/image and averaged) for evaluation of intra-observer variability.

**Statistical analysis**

Statistical analysis was performed using commercially available software$^{a,f}$. Normal distribution was visually assessed using histograms and QQ-plots. Normally distributed variables were presented as mean ± standard deviation, while non-normally distributed variables were presented as median (range). Pearson correlation was used to investigate a possible correlation between heart rate and MTG in healthy BT group. A paired t-test was used to compare the means of MVA$_{2D}$ and MVA$_{3D}$ for all the BT, while the Mann-Whitney U test was used to compare heart rate between healthy BT with normal MTG and increased MTG. Welch's ANOVA test followed by Games-Howell post-hoc analysis was used to compare normally distributed continuous variables between the healthy BT, BT with heart disease and healthy
boxers, while Kruskal-Wallis test followed by Dunn-Bonferroni post-hoc analysis was used to compare non-parametric continuous variables between groups. Agreement between MVA_{2D} and MVA_{3D} was evaluated using a Bland-Altman plot to visualize the comparisons graphically. Intra-and inter-observer variability for MVA_{2D} and MVA_{3D} were evaluated using the intra-class correlation coefficient based on an absolute-agreement, two-way random-effects model with type single. An intra-class correlation coefficient more than 0.8 indicated excellent agreement [29]. The significance level (alpha) was set to 0.05 for all analyses.

**Results**

A total of 71 BT were enrolled in our study. Serum biochemistry profiles of all dogs were within the in-house normal reference range. The MVA_{2D} was measurable in all BT, while MVA_{3D} was measurable for 69 BT. As a result, the two BT without MVA_{3D} measurements were excluded. Twenty BT were healthy, and 49 BT had heart disease. The BT group consisted of 23 males (33%) and 46 females (67%). The median age was 25 months (range: 11–138 months) and the mean body weight was 26.4 ± 3.4 kg (range: 20.0–34.2 kg). Heart diseases detected in the BT group are listed in Table A (data available in Supplemental Material on-line).

Heart rate was significantly different between healthy BT with normal MTG (n= 14, 125 beats per minute (bpm), range: 105–184 bpm) and healthy BT with increased MTG (>5 mmHg), (n= 5, 167 bpm, range: 153–189 bpm), (p = 0.02). Heart rate was significantly correlated with MTG in the healthy BT group (r = 0.8, n = 19, p <0.001).

All other echocardiographic variables were not different between groups.

A total of 15 healthy Boxers were included, consisting of eight males (53.3%) and seven females (46.7%) with a mean body weight of 29.6 ± 4.8 kg (range: 23.0–36.0
Demographic data and mitral valve area variables for each group are shown in Table 1. For all 69 BT (sick and healthy combined), the means of MVA\(_{2D}\) (2.9 ± 0.6 cm\(^2\)) and MVA\(_{3D}\) (2.1 ± 0.5 cm\(^2\)) were significantly different (p < 0.001), however, the means of MVA\(_{2D}\) and MVA\(_{3D}\) did not differ between healthy BT and BT with heart disease. All MVA variables for healthy BT and BT with heart disease were significantly lower (p < 0.05) than for healthy Boxers with no overlaps in MVA\(_{3D}\) between healthy BT and Boxers (Table 1).

The Bland-Altman plot of MVA\(_{2D}\) and MVA\(_{3D}\) revealed a wide limit of agreement, with a lower limit of 0.12 cm\(^2\) (95% confidence interval: 0.02–0.27 cm\(^2\)) and an upper limit of 1.5 cm\(^2\) (95% confidence interval: 1.4–1.7 cm\(^2\)). The mean difference was 0.82 ± 0.38 cm\(^2\) (95% confidence interval: 0.73–0.9 cm\(^2\); Fig. 3). The MVA\(_{2D}\) results were consistently higher than MVA\(_{3D}\). The intra-class correlation coefficient was excellent for both intra- and inter-observer variability for MVA\(_{2D}\) and MVA\(_{3D}\) (p < 0.001; Table 2).

Six BT were diagnosed with MS. Two-dimensional echocardiography showed incomplete diastolic leaflet separation, a restricted diastolic movement, thickened mitral valve leaflets, and a small mitral valve orifice. All six BT had MTG more than 5 mmHg. Using planimetry during this study, all six were confirmed with a small MVA\(_{3D}\) (<1.8 cm\(^2\)) relative to the healthy BT group. Demographic data and selected conventional echocardiographic variables for these BT are shown in Table 3. If MVA\(_{3D}\) less than 1.8 cm\(^2\) was used as the sole criterion for diagnosing MS, a total of 13 BT were diagnosed with MS.

**Discussion**

Mitral stenosis was diagnosed in 8.7% of BT using the MVA\(_{3D}\) less than 1.8 cm\(^2\) and MTG more than 5 mmHg. There was no overlap in MVA\(_{3D}\) between healthy BT and
Boxers, while MVA_{2D} value overlapped between these groups. This supports that MVA_{3D} can estimate the true narrowest orifice area in assessing dogs with MS in our study. This assertion was based on the feasibility and clinical utility, as the MVA is adjusted using multiplanar reconstruction and en-face cropping of the tip of the mitral valve without geometric assumption to assess the true mitral valve orifice [17,30]. In addition, both the intra- and inter-observer variability of MVA_{3D} were low and in accordance with those reported in human studies [17,21,31,32]. For the total 69 BT, the mean of MVA_{3D} was significantly lower than that of MVA_{2D}. This has also been reported in several studies in humans, although to a lesser degree than we report in the present study [16,21,31]. This finding implies that any deviation in the ideal image plane positioning will lead to consistently higher MVA_{2D} [33].

When MVA_{2D} and MVA_{3D} were evaluated separately, the mean MVA was not significantly different between healthy BT and BT with heart disease, as only six BT with MVD associated with MS were initially identified in this study. The group of BT with heart disease included phenotypes such as MVD, aortic stenosis and dilated cardiomyopathy, consistent with previous studies in BT and reflecting the complex cardiac phenotype of this breed [1].

According to the calculation for MVA_{2D} using the regression equation with the same BSA for different dog breeds [5], the means of MVA_{2D} indexed to BSA in healthy BT (3.3 ± 0.51 cm²) and healthy Boxers (4.5 ± 0.55 cm²) were lower than the mean of MVA_{2D} (5.5 ± 0.04 cm²) in this study. All BT in our study had a lower MVA_{2D} than the preliminary normal reference ranges previous published by O’Grady et al. (1986) [5]. Moreover, our study found that the means of MVA_{2D} indexed to BSA for healthy BT and BT with heart disease were significantly lower than for healthy Boxers. These
findings indicate that BT have a smaller MVA compared to Boxers which may suggest that BT have a smaller MVA in general than other breeds of dogs with similar BSA [5]. The BT have a higher risk of an early-onset progressive MVD with or without MS than other breeds, possibly due to a comparably smaller MVA. The mechanism behind the early-onset progressive MVD may be associated with flow-induced stress that activates cellular and molecular changes and leads to a superimposed tissue formation in the mitral valve [34].

Based on recommendations from the European Association of Echocardiography and the American Society of Echocardiography, the severity of MS should be evaluated using MVA planimetry, as the MTG depends on several factors such as heart rate and cardiac output [8]. In addition, it is recommended that MVA should be indexed to BSA, but no cut-off value is available for the indexed valve area to diagnose MS in humans [8].

Heart rate is the main contributor to an increased MTG in humans with MS [8]. Healthy BT with an increased MTG had significantly higher heart rate than healthy BT without an increased MTG, while there was no difference in MVA between the two groups. Furthermore, an increase in MTG was significantly correlated with an increased heart rate in healthy BT. These findings indicate that the heart rate is also the main contributor to an increased MTG in healthy BT.

Mitral valve motion, the peak velocity of early diastolic transmitral flow, and a prolonged pressure half-time on Doppler echocardiography without MVA planimetry are not ideal to diagnose MS [6]. High peak velocity of early diastolic transmitral flow indicates functional MS rather than anatomical stenosis [6], while several factors affect pressure half-time [9-13].
Mitral stenosis is difficult to diagnose in dogs as there were no cut-off values for MVA or MTG available. Therefore, preliminary reference ranges for MVA\textsubscript{2D} and MVA\textsubscript{3D} in Boxers was established in our study. These MVA ranges were comparable with preliminary reference ranges to the MVA\textsubscript{2D} ranges found in other dog breeds with a similar BSA [5]. The MVA values were also similar to the normal range of MVA\textsubscript{2D} found in humans (4-5 cm\textsuperscript{2}) [8]. The MVA\textsubscript{2D} and MVA\textsubscript{3D} measurements from BT indicated that all BT had some degree of MS as the mean for MVA\textsubscript{3D} in Boxers was 3.6 cm\textsuperscript{2} and the mean for MVA\textsubscript{3D} in BT was 2.1 cm\textsuperscript{2} with no overlap between groups. Then we established the smallest MVA\textsubscript{2D} and MVA\textsubscript{3D} in BT without echocardiographic evidence of cardiac abnormalities. The smallest MVA\textsubscript{3D} value in healthy BT was 1.8 cm\textsuperscript{2}. As a consequence, this value was subsequently used to define the diagnostic criteria for healthy BT. Six cases in our study had a low MVA\textsubscript{3D} measurement and other diagnostic criteria including a MVA\textsubscript{2D} less than 2.4 cm\textsuperscript{2}, increased MTG more than 6.6 mmHg, incomplete diastolic leaflet separation, a restricted diastolic movement and thickened mitral valve leaflets, thus suggesting a diagnosis of anatomical MS based on our initial criteria. The MVA\textsubscript{2D} values in these six cases were all lower than the MVA\textsubscript{2D} in a previous case report from a BT with MS [7]. The remaining seven BT with MVA\textsubscript{3D} measurements less than 1.8 cm\textsuperscript{2} and normal MTG were diagnosed with MS based on MVA\textsubscript{3D} alone. In humans, low-flow rate/low-MTG can be seen in severe MS associated with increased arterial afterload, subvalvular thickening and decreased left ventricular compliance [35].

This study did not apply Gorlin’s method, which has been considered to be the gold standard for measuring MVA in the past. However, Gorlin’s method is invasive and has several limitations such as requiring accurate measurements of oxygen...
consumption and transmitral gradients [36]. The MVA\textsubscript{3D} had the best agreement with Gorlin’s method compared with MVA\textsubscript{2D} and Doppler measurements, such as pressure half-time and flow convergence [16,18,31]. For these reasons, MVA\textsubscript{3D} is now considered the practical gold standard for diagnosing MS in humans [8].

The main limitation in our study for both MVA\textsubscript{2D} and MVA\textsubscript{3D} was the image quality during transthoracic scanning, with insufficient visualization of MVA. Furthermore, transthoracic two-dimensional echocardiography has several limitations. For example, the correct image plane orientation must be selected at the tips of the mitral valve leaflets [37]. Moreover, a skilled echocardiographer is needed, especially in dogs with a poor echo window or a distorted mitral valve [8]. The rate of failure to achieve a high-quality parasternal image was 17% in a previous report in humans [38]. Since the mitral valve apparatus is a complex saddle-shaped structure, it is better visualized by three-dimensional echocardiography imaging than two-dimensional echocardiography imaging. However, the spatial and temporal resolutions of current three-dimensional echocardiography technology are lower than for two-dimensional echocardiography [39]. Our control group consisted of a single dog breed, however, the Boxers in our study had a MVA\textsubscript{2D} similar to derived MVA values for dogs with a similar BSA [5]. As the intra-and inter-observer variability was evaluated by two of the authors, and not two randomly selected raters, we cannot exclude that the intra-class correlation coefficient is specific for our laboratory. Finally, the association of MVA to BSA was not validated. Instead, the index MVA to BSA was used to compare MVA\textsubscript{2D} between healthy BT and Boxers as well as derived MVA values for dogs with a similar BSA [5].

**Conclusion**
Mitrval valve area planimetry using two and three-dimensional echocardiography are feasible with a low observer variability. However, a poor agreement was found between MVA$_{2D}$ and MVA$_{3D}$ suggesting that these two methods cannot be applied interchangeably. Therefore, we prefer MVA$_{3D}$ for estimating the true narrowest orifice area in assessing dogs with MS. The smaller MVA in all BT compared to Boxers and dogs of similar BSA indicate some degree of MS.

**Conflict of Interest Statement**

The authors do not have any conflicts of interest to disclose.
Footnotes

³ GE Vivid E9 Ultrasonographic system, GE Healthcare, Brøndby, Denmark.
⁴ EchoPAC version 113 for the PC, GE Healthcare, Brøndby, Denmark.
⁵ IBM SPSS Statistics version 25, IBM, New York, United States.
⁶ MedCalc® Statistical software version 19.0.7., Ostend, Belgium.
References


[29] Landis JR, Koch GG. The measurement of observer agreement for categorical


Monaghan MJ. Impact of 3D echocardiography on grading of mitral stenosis


assessment of mitral valve stenosis by volumetric real-time three-dimensional


mitral valve area in mitral stenosis by real-time, three-dimensional

echocardiography versus two-dimensional echocardiography versus Doppler


[33] Johri AM, Passeri JJ, Picard MH. Three dimensional echocardiography:


[34] Kruithof BPT, Paardekooper L, Hiemstra YL, Goumans MJ, Palmen M,

Delgado V, Klautz RJM, Ajmone Marsan N. Stress-induced remodelling of the

mitral valve: a model for leaflet thickening and superimposed tissue formation


[35] El Sabbagh A, Reddy YNV, Barros-Gomes S, Borlaug BA, Miranda WR,

Pislaru SV, Nishimura RA, Pellikka PA. Low-Gradient Severe Mitral Stenosis:

Hemodynamic Profiles, Clinical Characteristics, and Outcomes. J Am Heart

Assoc 2019;8:e010736.

[36] Gorlin R, Gorlin SG. Hydraulic formula for calculation of the area of the

stenotic mitral valve, other cardiac valves, and central circulatory shunts. I. Am

Heart J 1951;41:1-29.


**Figure legends**

**Fig. 1** Mitral valve area planimetry using three-dimensional echocardiography (MVA\textsubscript{3D}) in an English Bull Terrier with mitral valve disease. (A) The translation was set at the smallest distance between the mitral valve leaflets and angled parallel to the mitral valve annulus. (B) Multiplanar reconstruction was used to locate the precise cross-sectional plane to cross the tips of all parts of the MV during the maximum early diastolic opening. (C) The constructed volume rendered image. (D) The constructed two-dimensional image was used to measure MVA.

AMVL: anterior mitral valve leaflet; LA: left atrium; PMVL: posterior mitral valve leaflet; MVO: mitral valve orifice.

**Fig. 2** Mitral valve area planimetry using two-dimensional (A) and three-dimensional (B and C) echocardiography in an English Bull Terrier with mitral stenosis. (A) shows a two-dimensional image used to measure MVA\textsubscript{2D}; note the MVA\textsubscript{2D} of 1.2 cm\textsuperscript{2}. (B) shows a three-dimensional image after multiplanar reconstruction, and (C) shows a two-dimensional image after multiplanar reconstruction to measure MVA\textsubscript{3D}; note the MVA\textsubscript{3D} of 0.9 cm\textsuperscript{2}.

AMVL: anterior mitral valve leaflet; IVS: interventricular septum; LVPW: left ventricular posterior wall; MVO: mitral valve orifice; PMVL: posterior mitral valve leaflet.

**Fig. 3** Bland-Altman comparisons of mitral valve area planimetry with two-dimensional (MVA\textsubscript{2D}) and three-dimensional (MVA\textsubscript{3D}) echocardiography in 69 English Bull Terrier. The middle line indicates the mean difference, and the upper and lower lines indicate the limits of agreement (mean ± 1.96 standard deviation).

cm\textsuperscript{2}: square centimeter; SD: standard deviation.
Table 1 Demographic data and mitral valve area planimetry variables using two-dimensional and three-dimensional echocardiography in healthy English Bull Terriers (BT), BT with heart disease, and healthy Boxers.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit of measurement</th>
<th>Healthy BT n = 20</th>
<th>BT with heart disease n = 49</th>
<th>Boxer n = 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>months</td>
<td>24.5 (12.0–71.0)</td>
<td>25 (11.0–138.0)</td>
<td>41.0 (13.0–102.0)</td>
</tr>
<tr>
<td>Weight</td>
<td>kg</td>
<td>25.8 ± 3.6 (20.0–32.5)</td>
<td>26.6 ± 3.4 (21.0–34.2)</td>
<td>29.6 ± 4.8 (23.0–36.0)</td>
</tr>
<tr>
<td>MVA&lt;sub&gt;2D&lt;/sub&gt;</td>
<td>cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>2.9 ± 0.46 (2.1–3.6)</td>
<td>2.9 ± 0.65 (1.3–4.1)</td>
<td>4.3 ± 0.62 (3.4–5.8)</td>
</tr>
<tr>
<td>MVA&lt;sub&gt;3D&lt;/sub&gt;</td>
<td>cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>2.1 ± 0.24 (1.8–2.7)</td>
<td>2.1 ± 0.53 (0.8–3.2)</td>
<td>3.6 ± 0.6 (2.9–4.6)</td>
</tr>
<tr>
<td>MVAI&lt;sub&gt;2D&lt;/sub&gt;</td>
<td>cm&lt;sup&gt;2&lt;/sup&gt;/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>3.3 ± 0.51 (2.3–4.6)</td>
<td>3.3 ± 0.76 (1.3–4.7)</td>
<td>4.5 ± 0.55 (3.9–6.0)</td>
</tr>
<tr>
<td>MVAI&lt;sub&gt;3D&lt;/sub&gt;</td>
<td>cm&lt;sup&gt;2&lt;/sup&gt;/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>2.4 ± 0.27 (2.0–3.0)</td>
<td>2.3 ± 0.61 (1.0–4.0)</td>
<td>3.7 ± 0.51 (2.9–4.8)</td>
</tr>
</tbody>
</table>

Normally distributed variables were presented as mean ± standard deviation (range), while non-normally distributed variables were presented as median (range). Welch’s ANOVA test followed by Games-Howell post-hoc analysis was used to compare normally distributed continuous variables between the healthy BT, BT with heart disease and healthy boxers, while Kruskal-Wallis test followed by Dunn-Bonferroni post-hoc analysis was used to compare non-normally distributed continuous variables between groups.

cm<sup>2</sup>: square centimeter; cm<sup>2</sup>/m<sup>2</sup>: square centimeter per square meter; kg: kilogram; MVA<sub>2D</sub>: mitral valve area measurement by planimetry using two-dimensional echocardiography; MVAI<sub>2D</sub>: mitral valve area measurement by planimetry using two-dimensional echocardiography indexed to body surface area; MVA<sub>3D</sub>: mitral valve area measurement by planimetry using three-dimensional echocardiography; MVAI<sub>3D</sub>: mitral valve area measurement by planimetry using three-dimensional echocardiography indexed to body surface area.

* Statistically significant difference (p < 0.001) compared with Boxers.
Table 2 Inter- and intra-observer variability of mitral valve area measurement by planimetry using two-dimensional and three-dimensional echocardiography.

<table>
<thead>
<tr>
<th></th>
<th>MVA$_{2D}$</th>
<th></th>
<th>MVA$_{3D}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICC 95%CI</td>
<td>p</td>
<td>ICC 95%CI</td>
</tr>
<tr>
<td>Inter observer variability</td>
<td>0.92 0.73–0.98 &lt;0.001</td>
<td>0.96 0.83–0.99 &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Intra observer variability</td>
<td>0.98 0.92–1.0 &lt;0.001</td>
<td>0.94 0.74–0.99 &lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

CI: confidence interval; ICC: intraclass correlation coefficient; MVA$_{2D}$: mitral valve area measurement by planimetry using two-dimensional echocardiography; MVA$_{3D}$: mitral valve area measurement by planimetry using three-dimensional echocardiography; p: significance level. Means of intra-class correlation coefficient based on an absolute-agreement, two-way random-effects model with type single.
### Table 3
Demographic data and selected conventional echocardiographic variables in six English Bull Terriers (BT) with mitral valve disease associated with mitral stenosis (BT1-BT6) and seven BT with MS based on \( \text{MVA}_{3D} \) less than 1.8 cm\(^2\) alone (BT7-BT13).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit of measurement</th>
<th>Healthy BT group</th>
<th>BT1</th>
<th>BT2</th>
<th>BT3</th>
<th>BT4</th>
<th>BT5</th>
<th>BT6</th>
<th>BT7</th>
<th>BT8</th>
<th>BT9</th>
<th>BT10</th>
<th>BT11</th>
<th>BT12</th>
<th>BT13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>-</td>
<td>-</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>Age (months)</td>
<td>24.5 (12.0–71.0)</td>
<td>23.0</td>
<td>31.2</td>
<td>24.0</td>
<td>31.0</td>
<td>28.3</td>
<td>25.6</td>
<td>26.5</td>
<td>21</td>
<td>22.5</td>
<td>31.5</td>
<td>23.4</td>
<td>27.5</td>
<td>30.3</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>25.8 ± 3.6 (20.0–32.5)</td>
<td>138.0 (12.0–71.0)</td>
<td>37.0</td>
<td>40.0</td>
<td>23.0</td>
<td>65.0</td>
<td>54.0</td>
<td>40.0</td>
<td>19.0</td>
<td>13.0</td>
<td>58.0</td>
<td>58.0</td>
<td>12.0</td>
<td>11.0</td>
<td></td>
</tr>
<tr>
<td>( \text{MVA}_{2D} ) (cm(^2))</td>
<td>2.9 ± 0.46 (2.1–3.6)</td>
<td>1.4</td>
<td>1.3</td>
<td>1.3</td>
<td>3.5</td>
<td>2.4</td>
<td>2.4</td>
<td>1.6</td>
<td>2.8</td>
<td>2</td>
<td>2.4</td>
<td>2.4</td>
<td>1.6</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>( \text{MVA}_{3D} ) (cm(^2))</td>
<td>2.1 ± 0.24 (1.8–2.7)</td>
<td>0.8</td>
<td>1.0</td>
<td>1.1</td>
<td>1.8</td>
<td>1.5</td>
<td>1.7</td>
<td>1</td>
<td>1.5</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
<td>1.7</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>( \text{MVAI}_{2D} ) (cm(^2)/m(^2))</td>
<td>3.3 ± 0.51 (2.3–4.6)</td>
<td>1.7</td>
<td>1.3</td>
<td>1.6</td>
<td>3.5</td>
<td>2.6</td>
<td>2.8</td>
<td>1.8</td>
<td>3.7</td>
<td>2.5</td>
<td>2.4</td>
<td>3.5</td>
<td>2.7</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>( \text{MVAI}_{3D} ) (cm(^2)/m(^2))</td>
<td>2.4 ± 0.27 (2.0–3.0)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.3</td>
<td>1.8</td>
<td>1.6</td>
<td>2.0</td>
<td>1.1</td>
<td>2.0</td>
<td>2.0</td>
<td>1.6</td>
<td>2.1</td>
<td>1.9</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>HR (CW) (bpm)</td>
<td>132 (105–189)</td>
<td>162</td>
<td>133</td>
<td>183</td>
<td>140</td>
<td>158</td>
<td>185</td>
<td>150</td>
<td>173</td>
<td>161</td>
<td>128</td>
<td>122</td>
<td>141</td>
<td>152(^a)</td>
<td></td>
</tr>
<tr>
<td>E (CW) (m/s)</td>
<td>1.2 (0.84–1.6)</td>
<td>-</td>
<td>1.9</td>
<td>1.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.3</td>
<td>1.3</td>
<td>1.4</td>
<td>1.2</td>
<td>1.3</td>
<td>1.6</td>
<td>1.1(^a)</td>
<td></td>
</tr>
<tr>
<td>EA fused (CW) (m/s)</td>
<td>-</td>
<td>3.1</td>
<td>-</td>
<td>2.0</td>
<td>2.0</td>
<td>2.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>MTG (CW) (mmHg)</td>
<td>3.3 (1.7–6.3)</td>
<td>23.3</td>
<td>8.4</td>
<td>6.7</td>
<td>6.6</td>
<td>9.6</td>
<td>18.7</td>
<td>4.2</td>
<td>4.5</td>
<td>5.2</td>
<td>2.6</td>
<td>3.6</td>
<td>6.1</td>
<td>3.2(^a)</td>
<td></td>
</tr>
<tr>
<td>PTG (CW) (mmHg)</td>
<td>5.6 (2.9–9.9)</td>
<td>39.1</td>
<td>13.9</td>
<td>12.0</td>
<td>16.3</td>
<td>16.5</td>
<td>27.6</td>
<td>6.5</td>
<td>6.6</td>
<td>8.1</td>
<td>5.4</td>
<td>6.7</td>
<td>9.7</td>
<td>5.2(^a)</td>
<td></td>
</tr>
<tr>
<td>AV Vmax (CW) (m/s)</td>
<td>1.9 (1.4–2.3)</td>
<td>3.1</td>
<td>7.2</td>
<td>2.2</td>
<td>2.2</td>
<td>5.1</td>
<td>1.8</td>
<td>2.8</td>
<td>2.0</td>
<td>2.7</td>
<td>1.7</td>
<td>2.4</td>
<td>1.9</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>PV Vmax (PW) (m/s)</td>
<td>1.1 (0.5–1.5)</td>
<td>0.62</td>
<td>1.7</td>
<td>1.4</td>
<td>1.0</td>
<td>2.2</td>
<td>1.4</td>
<td>1.7</td>
<td>1.3</td>
<td>1.5</td>
<td>1.0</td>
<td>1.3</td>
<td>1.2</td>
<td>1.3</td>
<td></td>
</tr>
</tbody>
</table>
Normally distributed variables were presented as mean ± standard deviation (range), while non-normally distributed variables were presented as median (range) for healthy BT group. 

E for BT1, BT4, BT5 and BT6: missing value due to fused mitral inflow.

AV Vmax: aortic peak velocity; cm²: square centimeter; bpm: beats per minute; cm²/m²: square centimeter per square meter; CW: continuous-wave Doppler; E: peak velocity of early diastolic transmitral flow; EA fused: peak velocity of fused mitral inflow; HR: heart rate; kg: kilogram; m/s: meter per second; mmHg: millimeter of mercury; MTG: mean transmitral gradient; MVA₂D: mitral valve area measurement by planimetry using two-dimensional echocardiography; MVA₁₂D: mitral valve area measurement by planimetry using two-dimensional echocardiography indexed to body surface area; MVA₃D: mitral valve area measurement by planimetry using three-dimensional echocardiography; MVA₁₃D: mitral valve area measurement by planimetry using three-dimensional echocardiography indexed to body surface area; PTG: peak transmitral gradients; PV V max: pulmonic peak velocity; PW: pulsed-wave Doppler.

Mitral inflow variables using PW.