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Effects of animal position on M-mode, two-dimensional, and Doppler echocardiographic measurements in healthy French bulldogs

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Abstract

Echocardiography is the most widely accepted diagnostic tool for assessment of cardiac function and morphology in dogs and is usually performed in lateral recumbency. However, in some situations or in stressed patients, it is necessary to perform it in a standing position. Only one study evaluated the effects of animal position on selected two-dimensional and M-mode echocardiographic variables in four healthy dogs of different breeds, but not in brachycephalic breeds. In these breeds echocardiographic evaluation is sometimes needed in standing position due to the severity of brachycephalic obstructive airway syndrome and the impossibility of managing them in lateral recumbency without causing stress and choking danger. The objectives of this prospective, observational study were to (a) evaluate the effects of lateral recumbency versus standing positions on echocardiographic M-mode, two-dimensional, Doppler flow measurements, and Tissue Doppler imaging in healthy French bulldogs (FBs); (b) assess the intra- and interoperator variability of the standing echocardiographic examination; and (c) compare the obtained results with the available data from the literature. Forty healthy FBs (20 females/20 males) were sampled. The median age and weight were 2.45 years (IQR_{25-75}, 1.18–4.16) and 12.7 kg (IQR_{25-75}, 10.88–13.46). There were no differences between lateral recumbency and standing position measurements ($P > 0.05$). Intraoperator coefficients of variation (CVs) ranged from 0.5% to 10.1%, whereas interoperator CVs ranged from 1% to 14.2%. Only E wave peak velocity, aortic, and pulmonary flows were consistent with the previously published reference ranges in lateral recumbency. In conclusion, echocardiography in a standing position could be a useful tool in FBs.

KEYWORDS
bouledogue français, echocardiography, lateral recumbency, standing position

ABBREVIATIONS: S', peak myocardial lateral velocity during systole; E', peak myocardial lateral velocity during early diastole.

1 | INTRODUCTION

Echocardiography is considered the most valuable diagnostic tool in the assessment of cardiac anatomy and function as well as cardiovascular diseases in small animal practice.[1,2] Present recommendations for performing complete echocardiographic studies in dogs include obtaining images in 2-dimensional (2D), M-mode, and Doppler modes. As reported by the echocardiographic guidelines, the lateral recumbency is recommended to decrease interference from the lungs.[3]

Practical reasons for performing examinations with the subject in lateral recumbency include ease of restraint by only one handler to facilitate collection of data and variation in the heights of standing dogs.[4] The height and the size of the dog can make it difficult for the operator to get correct scans, in particular without a table adjustable in height. Lateral recumbency allows obtaining repeatable examinations.[5] Published within- and between-day variability of conventional echocardiographic variables in standing cats and dogs show that both repeatability and reproducibility are good if the echocardiographers are suitably trained.[6,7] Performing the examinations with the subject in lateral recumbency has disadvantages: it may increase stress and may influence echocardiographic results in nervous/noncompliant dogs.[4] Moreover, some animals cannot tolerate a recumbent position because of discomfort or dyspnea, such as brachycephalic dogs (e.g., French and English bulldogs). The French bulldog (FB) is a small, brachycephalic breed. They have short and round bodies, with a large, deep, and full chest, which is often very difficult to evaluate with echocardiography; moreover, a large percentage are affected by severe brachycephalic airway obstruction syndrome (BOAS).[8–10]

We hypothesized that FBs are likely to have interchangeable echocardiographic measurements between standing and lateral recumbencies, with a very low intra- and interoperator variability. The purposes of this study were (a) to evaluate the effects of positioning (lateral recumbency vs. standing) on the echocardiographic M-mode, two-dimensional (2D), Doppler flow measurements, and tissue Doppler imaging (TDI) of the lateral mitral annulus in healthy FBs; (b) to assess the intra- and interoperator variability of the standing echocardiographic examination; (c) to compare the obtained results with the recent reference ranges proposed for the FBs and to propose reference ranges for the B-mode measurements of the left ventricle and the tricuspid annular plane systolic excursion (TAPSE), never published for this breed.[11]

2 | MATERIALS AND METHODS

2.1 | Selection and description of subjects

This study was a prospective, observational, and single-center study. French Bulldogs referred for a breed screening program were recruited from November 2021 to March 2022 at the Veterinary Medicine Department of the University of Milan. The sample size was based on convenience sampling. Informed consent was obtained from all the clients before starting any examination procedures for the included dogs, according to the Institutional Ethics Committee of the University of Milan. Owners were informed that the data obtained would be used for our research and all the owners accepted it. The Ethics Committee of the University of Milan excluded these criteria from the necessity of an ethical review (http://www.unimi.it/ateneo/20138.htm). The echocardiographic examinations were carried out as per the hospital's standard procedures. The 2D, M-mode, Doppler flow measurements (pulsed wave Doppler—PW), and TDI Doppler echocardiographic data obtained from lateral recumbency were compared with data in a standing position.

Inclusion criteria were as follows: certified FB breed, aged between 1 and 6 years, male or female, healthy, fit condition, owned, with no history of cardiac disease, showing no signs of illness during physical examinations, and no history of previous BOAS surgical correction. Included dogs were neither familiar with the examination procedure nor habituated to the operators; examinations were performed in the usual echocardiographic room. Only dogs with no structural or functional abnormalities in the standard M-mode, 2D, color Doppler, PW Doppler, and TDI echocardiographic examinations were included, as well as dogs showing electrocardiograms without conduction disturbances or ventricular/supraventricular arrhythmias.

All patients included in the study were evaluated according to the functional grading system developed by the Cambridge University group.[12] Each patient was then classified in one of the degrees of severity of BOAS following the evaluation of respiratory symptomatology before and after a 3-min exercise tolerance test at a trot at a speed of 4–5 km/h and defined as follows: free of respiratory signs (score 0), mild respiratory signs of BOAS which do not affect exercise tolerance (score 1), moderate respiratory signs of BOAS (the dog has a clinically relevant disease and requires management, including weight loss and/or surgical intervention) (score 2), and severe respiratory signs of BOAS (the dog should have a thorough veterinary examination with surgical intervention) (score 3).[12] Each dog was also evaluated for temperament according to the Maddern score as calm with playful attitude (score 1), slightly nervous and/or agitated (score 2), moderately nervous and/or agitated (score 3), and very nervous and/or agitated (score 4).[13] Only dogs with Cambridge scores between 0 and 2 and all temperament scores were admitted.[13] Dogs with Cambridge score 3 were not included because their breathing distress often made it difficult to perform echocardiographic examinations in lateral recumbency. The main purpose of this study was to evaluate the effects of positioning (lateral recumbency vs. standing) on the echocardiographic measurements in healthy FBs and for this reason, patients with BOAS Cambridge score 3 were excluded because only standing echocardiography was possible in these dogs. Moreover, the included dogs were cardiologically healthy. Severe BOAS can have circulatory repercussions on the right heart, increasing afterload and consequently, the functionality of the right heart can be modified.[14] For all these reasons dogs with Cambridge score 3 were not included in the study. Final decisions for subject inclusion/exclusion were made by two clinicians (P.G.B. and M.B.) with experience (nearly 25 and 9 years, respectively) in small animal cardiology (diagnosis and treatment and echocardiography), and by a surgeon with experience in small animal soft tissue surgery and particularly in canine brachycephalic airway syndrome (S.R.; nearly 25 years). The decisions were based on consensus.

2.2 Data recording and analysis

Lateral recumbency echocardiographic investigations were performed for all dogs by one clinician with 9 years of experience in small animal cardiology (M.B.). The operator knew the following general information at the time of echocardiography: breed, sex, health status, physical examination findings, vaccination information, castration status, and disease history.

Standard bipolar (I, II, and III) and unipolar (aVR, aVL, and aVF) lead electrocardiograms were recorded (Clickecg Cardioline, et medical devices Spa, Cavareno, Trentino, Italy) to detect cardiac arrhythmias and conduction disturbances. Following the physical examination, all dogs were shaved in the area on the right and left chest wall, where the heartbeat was palpable (fourth–sixth intercostal space). Echocardiographic studies were performed using a smart portable Doppler ultrasound device (Esaote, MyLab™ Omega, Genova, Italy) with multifrequency (1–5 MHz, 2–9 MHz) transducers and a simultaneous electrocardiographic tracing.[15] Echocardiographic examinations were performed in right and left standing and lateral positions in a dark and quiet room on a table with a cut-out. All dogs were not sedated during the examination. M-mode and 2D measurements were recorded according to the recommendations of the American Society of Echocardiography and in standing position as described by Chetboul et al.[4,15,16]

All valves were evaluated morphologically by 2D echocardiography. Left atrium (LA) and aortic root (Ao) diameters were measured in 2D echocardiography from the right parasternal short axis (RPSA) view at the level of the heart base in early diastole, and the left atrium to aorta ratio was calculated (LA:Ao).[17] The ratio between the Ao diameter and the early diastolic diameter of the pulmonary valve (PV) connecting the hinge points of the valve (Ao/PV) was evaluated in RPSA view.[18]

Left ventricular internal diameter (LVID), interventricular septum (IVS), and left ventricular posterior wall (LVPW) thickness in diastole (d) and systole (s) were acquired from the RPSA using a 2D-directed M-mode image at the level of the chordae tendineae with the beam directed perpendicular to the septum and then measured using a leading edge-to-leading edge method.[19,20] Left ventricular internal diameters in diastole and systole were indexed to the body surface area to provide the end-diastolic volume index (EDVI) and the end-systolic volume index (ESVI) with the Teicholz formula.[19,21–23] Moreover, LVIDd and LVIDs were indexed to body weight (normalized left ventricular internal diameter in diastole and systole, respectively, LVIDNd and LVIDNs).[20] End-diastolic volume (EDV) and end-systolic volume (ESV) were measured from the right parasternal long axis (RPLA) view using Simpson's method of disks.[24] Ejection fraction (EF) was calculated from volumes using the following formula: EF = [(EDV-ESV)/EDV] × 100.[20] Aortic valve annulus (AVa) and pulmonic valve annulus (PVa) diameters were measured using the inner edge to inner edge method (right below the insertion point of the valvular leaflets) from the RPLA view optimized for the left ventricular outflow tract and from the RPSA view at the level of the heart base, respectively, in early to mid-systole.[25] Aortic valve annulus was also measured from the left parasternal view optimized for the evaluation of the aortic root with open leaflets in early to mid-systole using the inner edge to inner edge method (AVa_{left}). The left parasternal cranial short axis view optimized for the evaluation of the pulmonary valve and the main pulmonary trunk was used to measure the PVa with open leaflets in early to mid-systole using the inner edge to inner edge method (PVa_{left}). The sphericity index (SI) was obtained by dividing the base-to-apex length at end-diastole measured from the RPLA using 2D echocardiography by the left ventricular internal diameter in diastole obtained from the same view.[5]

Blood flow velocities of the pulmonary artery were recorded with PW Doppler from the RPSA view with the sample volume placed within the artery just distal to the pulmonary valve. The peak blood flow velocity was measured from the PW recordings as the maximal value of the systolic flow signal. PW Doppler was employed also to record aortic blood flow velocities. Measurements were taken from the subcostal view. The sample volume was placed in the aorta just distal to the aortic valve. Maximal systolic flow signal readings obtained from the PW recordings were considered the peak blood flow velocity.[26] Velocity time integral and maximum peak velocity were measured from the aortic and pulmonic flow as previously described.[27]

Left ventricle inflow velocities were obtained with PW Doppler using the left parasternal apical four-chamber view, placing the sample volume in the ventricle at a depth corresponding to the tips of the mitral valve leaflets when wide open. Maximal early diastole (E) and late diastole (A) peak velocity flow signal readings obtained from the PW recordings were considered the peak blood flow velocities. A sample volume of 2–3 mm was set for all Doppler measurements.[26] In the TDI Doppler examination, the sample volume was set at the mitral annulus. Peak myocardial lateral velocities of mitral systole (S′), early diastole (E′), and late diastole (A′) were measured.[28]

Tricuspid annular plane systolic excursion (TAPSE) was obtained from an M-mode evaluation of the lateral part of the tricuspid valve annulus obtained with an "off-axis" left parasternal apical four-chamber view optimized for the right ventricle (RV), with the cursor carefully aligned parallel to the tricuspid longitudinal plane.[29] The same was performed for the mitral valve, measuring the mitralic annular plane systolic excursion (MAPSE) from a left parasternal apical four-chamber view with the M-mode method.[30] As described for the tricuspid valve, the cursor was carefully aligned parallel to the longitudinal displacement of the mitral valve plane with the left ventricular free wall. The mitral annular plane systolic excursion was measured using the leading-edge method as described for the TAPSE.[29]

Standing position operators' measurement variability was evaluated by repeated analysis of five randomly selected dogs. Operators were chosen for their similarly high levels of experience in echocardiographic imaging. The studies were performed three times within 4 weeks by one operator (M.B.) for the determination of intraoperator measurement variability and one time by two operators (M.B. and C.L.) for the determination of interoperator measurement variability;

measurements made by the observers were unknown to each other. To exclude the influence of a measurement on the following one, the operators were prevented from seeing the values of the obtained measurements on the ultrasound screen.

2.3 | Statistics

Statistical analyses were performed by a veterinary clinician (M.B.) with statistics training and supervised by a statistician using commercially available statistics software (SPSSTM 27.0, IBM, SPSS, USA). Descriptive statistics were generated. The distribution of data for continuous variables was assessed for normality by means of the Kolmogorov–Smirnov test. Variables were not normally distributed, and results were reported as median and interquartile ranges ($IQR_{25–75}$) unless otherwise specified. A Mann–Whitney U test was used to compare median echocardiographic values between males and females. A Wilcoxon test for paired samples was used to compare median echocardiographic values between lateral and standing positions. A Spearman correlation coefficient (*r*) was applied to study the correlation between nostril grading and temperament scores and echocardiographic measurements. The correlation was considered weak, moderate, strong, or perfect respectively when the value of the correlation coefficient was 0.1–0.3, 0.4–0.6, 0.7–0.9, or 1.[31] The main echocardiographic variables used in clinical practice were compared with previously published reference intervals in FBs obtained in lateral recumbency using a Mann–Whitney U test.[11] Intra- and interoperator measurement variability for different echocardiographic variables was calculated using the coefficient of variation (CV) and the following formula: CV (%) = (mean difference of the measurements/average of the measurements) × 100. The degree of variability was classified as previously reported: CV < 15% (good), and CV ≥ 15% (poor).[4] A *P*-value < 0.05 was considered significant for all analyses.

3 | RESULTS

Out of the 51 candidates, 11 FBs were excluded from the study because of the impossibility to maintain lateral recumbence positioning and/or anamnesis of BOAS surgical correction. Forty FBs were included in the study, with 20 females (three spayed females, 7.5%) and 20 males (1 neutered male, 2.5%). The median age was 2.45 years ($IQR_{25–75}$, 1.18–4.16), and the median weight was 12.7 kg ($IQR_{25–75}$, 10.88–13.46). The mean heart rate was 124.4 beats per minute (bpm) (range, 61–178 bpm). Descriptive clinical data are reported in Table 1.

In four dogs (10%), it was not possible to evaluate left ventricular volumes from the RPLA view because of the suboptimal images with no clear definition of the endocardial border especially on the apex of the left ventricle. In two dogs (5%), it was not possible to evaluate the trans-mitral flow pattern because of the fusion of the E and A waves due to high heart rates (>150 bpm), the same was observed for the TDI measurements in the same subjects.

TABLE 1 Clinical characteristics of the included 40 healthy French bulldogs.

	Overall population				
	Median	IQR_{25}	IQR_{75}	SD	Mean
Weight (kg)	12.70	10.88	13.46	2.11	12.11
BSA (m^2)	0.55	0.50	0.57	0.06	0.53
Age	2.45	1.18	4.16	2.00	2.82
Neck circumference (cm)	38.00	35.50	41.00	4.72	38.49
Neck length (cm)	14.00	13.00	16.50	3.76	14.95
Thoracic circumference (cm)	55.50	52.50	58.00	7.96	54.51
BCS	6.00	5.00	6.00	0.74	5.92
Temperament	21 dogs score 1 (52.5%) 12 dogs score 2 (30%) 7 dogs score 3 (17.5%)				
Cambridge classification	4 dogs score 0 (10%) 24 dogs score 1 (60%) 12 dogs score 2 (30%)				

Males were heavier and older than females (*P* = 0.001 and *P* = 0.02, respectively) and had greater LV diameters in 2D and M-mode measurements, both in systole and diastole (*P* < 0.05). The same was observed for the left ventricular mass (*P* = 0.02). Furthermore, males had greater aortic and pulmonary annulus, both in the right (*P* = 0.03 and *P* = 0.01, respectively) and left parasternal views (*P* = 0.01 and *P* = 0.03, respectively).

The 2D, M-mode, PW, and TDI echocardiographic measurements obtained both in lateral recumbency and standing position are reported in Tables 2–4 divided according to the positioning (lateral recumbency vs. standing).

There were no differences between the positioning (lateral recumbency vs. standing position) for all the evaluated echocardiographic variables as indicated in Tables 2–4 (all *P* > 0.05).

The observers reported a subjectively better visualization of the pulmonary annulus and its Doppler flow from the left parasternal cranial short-axis view optimized for the evaluation of the pulmonary valve and the main pulmonary trunk with the dog in a standing position compared with the same view obtained in lateral recumbency (Figure 1).

Intraoperator CVs of the echocardiographic variables measured in the standing positions were summarized in Tables 2–4, as well as the interoperator CVs. Values for intra-CVs ranged from 0.5% to 10.1%, whereas inter-CVs ranged from 1% to 14.2% (Table 5).

Data comparison between the echocardiographic values in the present study and previously published values in this breed showed that all the obtained results did not differ from the literature (*P* > 0.05).

4 | DISCUSSION

This is the first published study comparing echocardiographic measurements in FBs that were considered healthy based on unremarkable

TABLE 2 Two-dimensional echocardiographic values in the included 40 healthy French bulldogs, divided also according to the recumbency.

	Overall population					Lateral recumbency					Standing					
	Median	IQR_{25}	IQR_{75}	SD	Mean	Median	IQR_{25}	IQR_{75}	SD	Mean	Median	IQR_{25}	IQR_{75}	SD	Mean	*P*-value
LVdA (cm^2)	9.31	7.88	10.35	2.03	9.36	9.47	8.38	11.38	2.04	9.76	9.28	7.77	10.10	9.76	1.98	0.53
LVdL (cm)	3.98	3.62	4.24	0.51	3.97	4.07	3.68	4.32	0.56	4.05	3.92	3.55	4.24	4.05	0.47	0.65
EDV (cm^3)	18.55	14.63	21.73	6.17	18.55	19.20	16.08	24.73	5.93	19.58	17.05	14.08	20.75	19.58	6.32	0.46
LVsA (cm^2)	3.63	2.89	4.33	1.06	3.63	3.88	2.93	4.58	1.22	3.85	3.43	2.92	4.06	3.85	0.88	0.38
LVsL (cm)	2.76	2.51	3.09	0.41	2.80	2.90	2.58	3.25	0.48	2.90	2.69	2.47	3.02	2.90	0.34	0.43
ESV (cm^3)	3.82	2.73	5.28	1.96	4.12	4.66	2.73	5.70	2.18	4.49	3.56	2.75	4.58	4.49	1.73	0.23
EF (%)	78.20	73.00	84.30	8.43	77.84	78.50	72.25	83.45	8.53	77.76	78.20	76.10	84.60	77.76	8.47	0.53
SV (cm^3)	6.91	3.69	15.70	7.54	9.90	8.75	3.46	14.30	7.22	9.55	5.54	3.75	17.00	9.55	7.90	0.42
AVa_{left} (cm)	1.26	1.20	1.33	0.14	1.28	1.27	1.20	1.32	0.15	1.28	1.26	1.19	1.34	1.28	0.13	0.61
AVa (cm)	1.21	1.15	1.29	0.13	1.21	1.20	1.16	1.29	0.13	1.21	1.22	1.15	1.29	1.21	0.12	0.57
PVa_{left} (cm)	1.38	1.30	1.48	0.12	1.39	1.39	1.33	1.49	0.11	1.40	1.38	1.28	1.47	1.40	0.13	0.64
PVa (cm)	1.31	1.25	1.37	0.10	1.31	1.31	1.22	1.36	0.10	1.29	1.31	1.27	1.37	1.29	0.11	0.46
Ao (cm)	1.50	1.39	1.63	0.17	1.51	1.48	1.36	1.62	0.16	1.48	1.51	1.42	1.64	1.48	0.17	0.59
LA (cm)	1.81	1.68	1.94	0.24	1.84	1.76	1.66	1.94	0.25	1.83	1.83	1.72	1.95	1.83	0.23	0.6
LA:Ao	1.20	1.14	1.30	0.12	1.23	1.24	1.15	1.32	0.14	1.24	1.19	1.14	1.29	1.24	0.11	0.79
PV (cm)	1.44	1.32	1.59	0.23	1.45	1.44	1.41	1.65	0.26	1.51	1.40	1.32	1.55	0.20	1.40	0.57
Ao:PV	1.04	0.96	1.15	0.15	1.05	0.98	0.89	1.04	0.17	1.00	1.08	0.98	1.17	0.13	1.08	0.58
SI	1.49	1.23	1.59	0.24	1.53	1.47	1.32	1.52	0.21	1.45	1.51	1.45	1.54	0.14	1.49	0.53

Abbreviations: Ao, aorta diameter; Ao:PV, aorta to pulmonary artery ratio; AVa, aortic valve annulus; AVa_{left}, aortic valve annulus left apical view; EDV, end-diastolic left ventricular volume; EF, ejection fraction; ESV, end-systolic left ventricular volume; LA, left atrium diameter; LA/Ao, left atrium to aorta ratio; LVdA, left ventricular diastolic area; LVdL, left ventricular diastolic length; LVsA, left ventricular systolic area; LVsL, left ventricular systolic area; PV, pulmonary valve diameter; PVa, pulmonic valve annulus; PValeft, pulmonic valve annulus left apical view; SI, sphericity index. The mean and standard deviation (SD) are presented for all data, as median and interquartile ranges (25th–75th percentile).; SV, stroke volume.

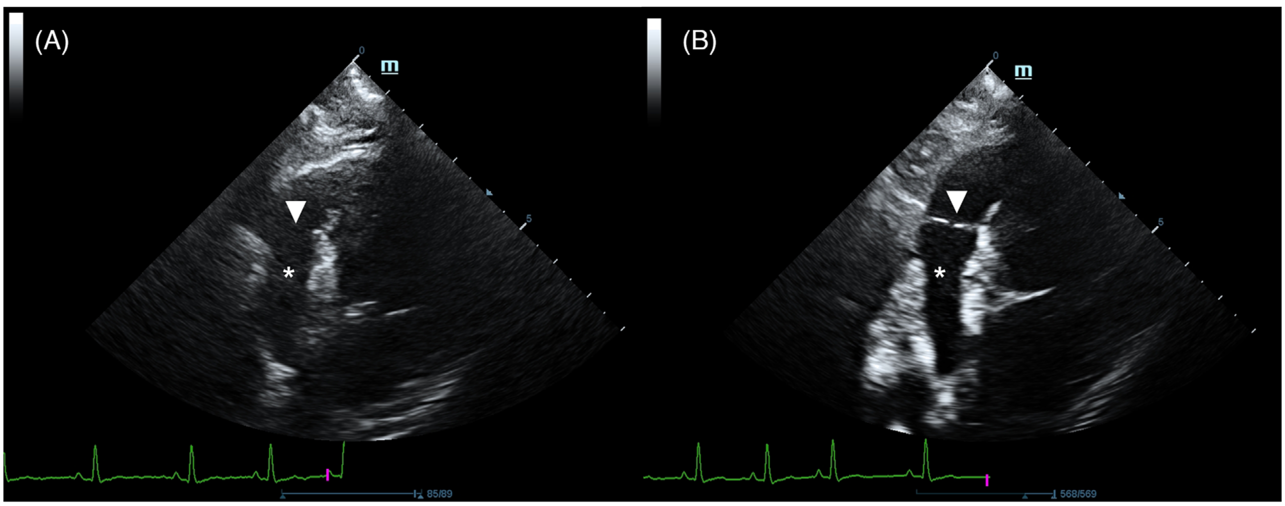

FIGURE 1 B-mode images obtained by Mindray Vetus 7 machine with a multifrequency (7–3 MHz) phased array probe from the cranial left short axis view optimized for evaluation of the pulmonary artery (*) and pulmonary valve (arrowhead) in lateral recumbency (A) and standing position (B) at end-diastole (closed leaflets) in a male French Bulldog (3 years, 11 kg) affected by BOAS Cambridge score 1. The images highlight that the standing position allows for optimizing and improving the evaluation of the main pulmonary artery and pulmonary valve. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 3 M-mode echocardiographic values in the included 40 healthy French bulldogs, divided also according to the recumbency.

	Overall population					Lateral recumbency					Standing					
	Median	IQR_{25}	IQR_{75}	SD	Mean	Median	IQR_{25}	IQR_{75}	SD	Mean	Median	IQR_{25}	IQR_{75}	SD	Mean	*P*-value
IVSd (mm)	8.60	7.62	9.30	1.42	8.52	8.30	7.24	9.32	1.54	8.45	8.62	7.80	9.30	8.45	1.34	0.54
LVIDd (mm)	29.50	27.18	31.83	3.95	29.17	29.50	26.60	31.90	4.55	28.95	29.50	27.30	31.10	28.95	3.43	0.46
LVPWd (mm)	8.50	7.40	9.31	1.52	8.60	8.60	7.69	9.59	1.41	8.69	8.31	7.39	9.30	8.69	1.62	0.59
IVSs (mm)	10.90	9.50	11.93	1.66	10.89	10.50	9.27	11.45	1.45	10.45	11.20	10.20	12.80	10.45	1.75	0.71
LVIDs (mm)	17.10	14.80	18.65	3.12	16.81	17.90	15.60	18.70	3.06	17.18	16.70	14.50	17.90	17.18	3.18	0.26
LVPWs (mm)	12.00	11.20	12.93	1.68	12.24	12.00	11.20	12.65	1.73	12.14	12.10	11.20	13.20	12.14	1.66	0.63
EF (%)	74.55	68.48	79.33	8.08	74.19	73.00	68.95	76.10	8.01	71.91	77.30	68.60	83.40	71.91	7.73	0.06
FS (%)	42.00	37.00	47.70	8.07	42.14	41.00	37.50	44.00	7.49	40.91	44.00	37.00	50.00	40.91	8.48	0.27
LV mass (g)	65.00	54.25	76.75	19.12	66.77	61.50	53.50	77.75	18.24	65.29	65.50	56.25	73.75	65.29	20.36	0.80
ESVI	16.40	10.83	19.16	6.97	16.19	17.58	11.73	19.48	6.84	17.00	14.56	10.63	18.08	17.00	7.09	0.54
EDVI	63.89	53.49	74.45	16.73	63.01	64.86	51.17	74.77	17.61	61.95	62.91	54.60	71.73	61.95	16.15	0.41
LVIDNs	0.83	0.71	0.88	0.14	0.81	0.85	0.73	0.89	0.14	0.82	0.79	0.70	0.86	0.82	0.14	0.51
LVIDNd	1.42	1.32	1.52	0.17	1.40	1.43	1.30	1.52	0.20	1.39	1.41	1.33	1.49	1.39	0.15	0.41
EPSS (cm)	0.27	0.23	0.31	0.06	0.27	0.27	0.23	0.30	0.05	0.26	0.27	0.23	0.31	0.26	0.06	0.50
MAPSE (cm)	1.02	0.87	1.21	0.23	1.05	1.02	0.90	1.16	0.23	1.03	1.05	0.87	1.23	1.03	0.23	0.28
TAPSE (cm)	1.09	0.90	1.29	0.26	1.09	1.03	0.88	1.25	0.24	1.04	1.09	0.92	1.32	1.04	0.27	0.07

Abbreviations: EDVI, end-diastolic volume index; EF, ejection fraction; EPSS, E-point to septal separation; ESVI, end-systolic volume index; FS, fractional shortening; IVSd, interventricular septal thickness at end-diastole; IVSs, interventricular septal thickness at end-systole; LV mass, left ventricular mass; LVIDd, left ventricular internal dimension at end-diastole; LVIDNd, normalized left ventricular internal diameter in diastole; LVIDNs, normalized left ventricular internal diameter in systole; LVIDs, left ventricular internal dimension at end-systole; LVPWd, left ventricular posterior wall thickness at end-diastole; LVPWs, left ventricular posterior wall thickness at end-systole; MAPSE, mitral annular plane systolic excursion; SI, sphericity index; TAPSE, tricuspid annular plane systolic excursion. The mean and standard deviation (SD) are presented for all data, as median and interquartile ranges (25th–75th percentile).

history and normal physical examination, except for BOAS from Cambridge score 0 to 2, six-lead electrocardiography, and transthoracic echocardiography obtained both in lateral recumbency and standing position. Chetboul et al.[4] demonstrated no differences between echocardiographic measurements obtained in the two positions, but currently there is no study performed on a single breed and none on a brachycephalic breed. The excellent obtained results indicate that the use of the standing position yields the same results and provides good intra and interoperator CVs for all variables. These data agree with the results of previous echocardiographic studies performed in awake-standing dogs and cats.[4,9,10] In addition, the standing technique was possible in all included subjects (100% feasibility).

Only FBs were included in this study, consequently, different results might be obtained with other breeds. According to the authors' opinion, because of breed-specific morphologic characteristics of the thorax, for some breeds, the standing position might be more suitable than the lateral recumbency for the echocardiographic examination. The morphology of the breed, the barrel chest, a low height at the withers, and the short neck made it suitable for the evaluation in a standing position, making them easy to contain, with a minimum movement of the neck and the thorax. For example, it is possible that in dogs with a larger thorax, such as English Bulldogs, higher-quality images might be obtained with the dog in lateral recumbency rather than the standing position. The same may occur for a dolichocephalic dog or a large-sized dog if a height-adjustable echocardiographic table is not available. However, it should be highlighted that the authors have better visualized and measured the pulmonary annulus and its Doppler flow from the left parasternal cranial short-axis view optimized for the evaluation of the pulmonary valve and the main pulmonary trunk with the dog in a standing position compared with the same view obtained in lateral recumbency, maybe for a better chance of avoiding the scapular muscles and the lung. This suggests the possibility of better evaluating this area in a standing position in dogs affected by pulmonary stenosis.

Echocardiographic reference ranges for M-mode and Doppler flows measurements in FBs have been published by Vurucu et al.[11] The echocardiographic data of the included population of the present study did not statistically differ from the published data.[11] However, some differences could exist between the included populations such as the morphology and somatotype of the dogs, also among the same brachycephalic breed.[11,20,23] The morphology of the FBs may vary between Countries: in our study, only subjects that comply with the breed standard were included, whereas Vurucu et al.[11] did not state this aspect. Eventually, Vurucu et al.[11] did not classify the BOAS of the included FBs according to the Cambridge scale. For this reason, it is not known if the included dogs were affected by BOAS and in which gravity score. Regarding the normalized LVIDd, the values found in the present study (1.40 ± 1.17 for the general population) were in line with the previous study on canine general population.[20,23] However, the study by Cornell

TABLE 4 Doppler echocardiographic values in the included 40 healthy French bulldogs, divided also according to the recumbency.

	Overall population					Lateral recumbency					Standing					
	Median	IQR_{25}	IQR_{75}	SD	Mean	Median	IQR_{25}	IQR_{75}	SD	Mean	Median	IQR_{25}	IQR_{75}	SD	Mean	*P*-value
AoVTI (m)	0.12	0.11	0.13	0.02	0.12	0.13	0.12	0.14	0.02	0.13	0.11	0.10	0.13	0.13	0.02	0.78
ApV (m/s)	1.16	1.03	1.36	0.32	1.22	1.20	1.09	1.31	0.34	1.23	1.11	0.99	1.39	1.23	0.31	0.67
ApG (mm Hg)	5.40	4.28	7.00	2.61	6.17	6.00	4.93	7.00	2.36	6.38	5.00	4.00	7.00	6.38	2.81	0.75
ET (ms)	165.00	156.75	177.25	19.47	165.10	169.00	162.00	175.00	12.19	171.23	159.00	146.00	178.00	171.23	22.84	0.52
PaVTI (m)	0.11	0.10	0.13	0.02	0.11	0.12	0.10	0.13	0.03	0.11	0.11	0.10	0.13	0.11	0.02	0.97
PpV (m/s)	1.02	0.85	1.20	0.28	1.01	1.02	0.85	1.13	0.24	1.02	1.02	0.85	1.21	1.02	0.30	0.99
PpG (mm Hg)	4.00	3.00	6.00	2.03	4.53	4.00	3.00	5.00	2.19	4.41	4.10	3.00	6.00	4.41	1.92	0.89
E (m/s)	0.75	0.66	0.83	0.14	0.75	0.73	0.66	0.83	0.15	0.76	0.77	0.68	0.82	0.76	0.13	0.72
A (m/s)	0.61	0.54	0.70	0.15	0.64	0.62	0.54	0.69	0.14	0.64	0.60	0.54	0.71	0.64	0.15	0.99
E/A	1.20	1.10	1.40	0.25	1.22	1.20	1.10	1.38	0.21	1.21	1.25	1.02	1.40	1.21	0.28	0.99
MVEdc (ms)	48.00	41.00	57.00	10.50	48.25	51.00	41.00	57.00	10.37	49.85	45.00	39.50	52.50	49.85	10.78	0.86
MVEacc (ms)	141.00	109.00	152.00	29.88	131.81	125.00	103.75	150.00	33.15	129.83	145.50	111.50	152.00	129.83	27.93	0.71
E'sept (m/s)	0.20	0.09	0.32	0.19	0.24	0.16	0.10	0.30	0.21	0.23	0.25	0.09	0.34	0.23	0.17	0.68
A'sept (m/s)	0.16	0.07	0.27	0.15	0.18	0.12	0.07	0.26	0.17	0.18	0.17	0.07	0.27	0.18	0.13	0.58
S' (m/s)	0.39	0.36	0.44	0.08	0.39	0.41	0.36	0.45	0.09	0.39	0.39	0.35	0.44	0.39	0.07	0.83

Abbreviations: A, mitral valve peak velocity of late transmitral flow; A'sept, late diastolic motion wave recorded at the septal aspect of the mitral annulus by pulsed-wave tissue doppler imaging; AoVTI, aortic velocity-time integral; ApG, aortic valve peak gradient; ApV, aortic valve peak velocity; E, mitral valve peak velocity of early diastolic transmitral flow; E/A, mitral valve ratio of E to A; E'sept, early diastolic motion wave recorded at the septal aspect of the mitral annulus by pulsed-wave tissue doppler imaging; ET, aortic ejection time; MVEacc, mitral valve early diastolic flow acceleration time; MVEdc, mitral valve E velocity deceleration time; PaVTI, pulmonary artery velocity time integral; PpG, pulmonary valve peak gradient; PpV, pulmonary valve peak velocity; S', systolic tissue velocities. The mean and standard deviation (SD) are presented for all data, as median and interquartile ranges (25th–75th percentile).

et al.[20] identified a normalized LVIDd upper limit of 1.85. This difference could be due to the large population of Irish wolfhounds (29% of the cohort) included in the study by Cornell et al.[20]

This is the first study that reports echocardiographic reference ranges for left ventricular 2D measurements and TAPSE for this breed.

Regarding left ventricular volumes, our results suggest a smaller EDV index (63.0 ± 16.73 mL/m^2 for the general population) and ESV index (16.19 ± 6.97 mL/m^2 for the general population) and a higher EF (74.19 ± 8.08% for the general population) in FBs than those in Boxers (71 ± 11 mL/m^2, 36 ± 7 mL/m^2, 49 ± 7%),[32] Doberman (38 ± 6 ml/m^2, 72 ± 8 mL/m^2, 49 ± 6%),[20] and more similar to those reported for English Bulldogs (46.8 ± 9.9 mL/m^2, 16.4 ± 5.5 mL/m^2, 65.1 ± 9.1%).[32] A SI less than 1.65 has been proposed as a cutoff to diagnose systolic dysfunction and has been considered as a minor criterion for the diagnosis of dilated cardiomyopathy in dogs; however, its clinical value has been questioned because of its low sensitivity and specificity compared with volume-based parameters.[5] Furthermore, a reference range for dogs not predisposed to cardiomyopathies, for small breed dogs or dogs with brachycephalic morphology, except for English Bulldogs,[33] has never been proposed. In our population of healthy FBs, the SI was under the reference limit of 1.65, with a mean value of 1.49. One reason for this difference could be the breed-specific cardiac morphology and geometry of the FB, which based on our results have a more rounded heart compared with other breeds.

The first limitation of the study is certainly that the interoperator CV has been calculated between experienced operators. We cannot claim to achieve the same results for operators with lower levels of expertise. Furthermore, the operators who performed the measurements and variability tests in this work were used to standing evaluation. Second, the sample size is small compared with the usually recommended sample size of 120 dogs, and the study was performed in a single veterinary center.[22,34] Third, we did not evaluate right ventricular echocardiographic variables, and future studies are needed to evaluate the right heart dimension and function in this breed. The last limit is lack of the evaluation of the influence of the functional grading system (Cambridge classification) of BOAS and the temperament score on the feasibility of echocardiographic examination in lateral recumbency, especially in subjects with grade 3 BOAS. It would be interesting to evaluate if the temperament of the dog, rather than the severity of BOAS or their combination, can exacerbate respiratory symptoms (dyspnea, stertor, stridor, and/or cyanosis) during lateral containment and if there are differences between echocardiographic data obtained in standing position in dogs with different grading scores. For this purpose, further studies including a greater number of subjects are needed.

In conclusion, this study demonstrated no differences in the echocardiographic measurements obtained in lateral recumbency and standing position in a sample of FBs, excluding subjects with severe respiratory signs of BOAS (Cambridge classification grade 3). Authors recommend that small animal cardiology clinicians gain experience in standing echocardiographic examination to be able to obtain optimal evaluations in stressed or dyspneic FBs affected by BOAS. Further

TABLE 5 Results showing intraoperator (CV intra) and interoperator (CV inter) coefficient of variation.

B-mode echocardiographic values	CV intra	CV inter	M-mode echocardiographic values	CV intra	CV inter	Doppler echocardiographic values	CV intra	CV inter
LVdA (cm^2)	1.00	11.12	IVSd (mm)	1.61	3.21	AoVTI (m)	7.3	8.97
LVdL (cm)	1.62	8.110	LVIDd (mm)	1.02	3.52	ApV (m/s)	3.0	13.87
EDV (cm^3)	1.53	13.23	LVPWd (mm)	1.63	12.31	ApG (mmHg)	3.6	13.94
LVsA (cm^2)	4.81	3.21	IVSs (mm)	1.92	8.09	ET (ms)	1.3	4.42
LVsL (cm)	1.95	11.30	LVIDs (mm)	1.34	6.11	PaVTI(m)	6.8	14.19
ESV (cm^3)	2.74	3.05	LVPWs (mm)	0.92	6.80	PpV (m/s)	2.4	7.86
EF (%)	0.91	9.13	EF (%)	0.81	5.07	PpG (mmHg)	1.8	12.97
SV (cm^3)	1.66	4.17	FS (%)	0.92	6.13	E (m/s)	4.7	8.22
AVa_{left} (cm)	2.27	6.40	LV mass (g)	10.11	10.03	A (m/s)	4.2	11.34
AVa (cm)	3.63	6.91	ESVI	0.76	4.22	E/A	1.8	13.04
PVa_{left} (cm)	3.39	7.09	EDVI	0.52	4.06	MVEdc (ms)	1.2	7.83
PVa (cm)	3.05	8.34	LVIDNs	0.67	4.14	MVEacc (ms)	0.9	0.00
Ao (cm)	2.84	1.28	LVIDNd	0.61	2.97	E'sept (m/s)	5.3	13.92
LA (cm)	2.12	4.20	EPSS (cm)	5.13	3.89	A'sept (m/s)	6.0	13.44
LA:Ao	2.00	3.10	MAPSE (cm)	3.78	12.10	S' (m/s)	8.2	7.32
PV (cm)	2.40	3.22	TAPSE (cm)	4.49	10.05			
Ao:PV	2.76	2.94						

Abbreviations: A′sept, late diastolic motion wave recorded at the septal aspect of the mitral annulus by pulsed-wave tissue doppler imaging; Ao:PV, aorta to pulmonary artery ratio; AoVTI, aortic velocity-time integral; ApG, aortic valve peak gradient; ApV, aortic valve peak velocity; AVa, aortic valve annulus; AVa_{left}, aortic valve annulus left apical view; B-Mode: Ao, aorta diameter; E, mitral valve peak velocity of early diastolic transmitral flow; E/A, mitral valve ratio of E to A; E′sept, early diastolic motion wave recorded at the septal aspect of the mitral annulus by pulsed-wave tissue doppler imaging; EDV, end diastolic left ventricular volume; EF, ejection fraction; EF, ejection fraction; EPSS, E-point to septal separation; ESV, end systolic left ventricular volume; ESVI, end systolic volume index; ET, aortic ejection time; FS, fractional shortening; IVSd, interventricular septal thickness at end-diastole; IVSs, interventricular septal thickness at end-systole; LA, left atrium diameter; LA/Ao, left atrium to aorta ratio; LV mass, left ventricular mass; LVdA, left ventricular diastolic area; LVdL, left ventricular diastolic length; LVIDd, left ventricular internal dimension at end-diastole; LVIDNd, normalized left ventricular internal diameter in diastole; LVIDNs, normalized left ventricular internal diameter in systole; LVIDs, left ventricular internal dimension at end-systole; LVPWd, left ventricular posterior wall thickness at end-diastole; LVPWs, left ventricular posterior wall thickness at end-systole; LVsA, left ventricular systolic area; LVsL, left ventricular systolic area; MAPSE, mitral annular plane systolic excursion; MVEacc, mitral valve early diastolic flow acceleration time; MVEdc, mitral valve E velocity deceleration time; PaVTI, pulmonary artery velocity time integral; PpG, pulmonary valve peak gradient; PpV, pulmonary valve peak velocity; PV, pulmonary valve diameter; PVa, pulmonic valve annulus; PVa_{left}, pulmonic valve annulus left apical view. M-mode: EDVI, end diastolic volume index; S′, systolic tissue velocities.; SV, stroke volume; TAPSE, tricuspid annular plane systolic excursion. Doppler: A, mitral valve peak velocity of late transmitral flow.

studies are needed to evaluate the result of the present study in a larger cohort of dogs and in different brachycephalic breeds. However, we believe that our results may be useful for proper echocardiographic interpretation and screening purposes in this widespread and fashionable breed. The obtained results can be a valuable help for small animal cardiology clinicians who have to manage these patients.

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LIST OF AUTHOR CONTRIBUTIONS

Category 1

a) Conception and design: Bagardi, Romussi, Locatelli, Brambilla, Ghilardi.
b) Acquisition of data: Bagardi.
c) Analysis and interpretation of data: Bagardi, Locatelli.

Category 2

a) Drafting the article: Bagardi, Locatelli.
b) Revising article for intellectual content: Bagardi, Romussi, Locatelli, Brambilla, Ghilardi.

Category 3

a) Final approval of the completed article: Bagardi, Romussi, Locatelli, Brambilla, Ghilardi.

Category 4

a) 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: Bagardi, Romussi, Locatelli, Brambilla, Ghilardi.

CONFLICT OF INTEREST STATEMENT

The authors declare that there were no conflicts of interest.

PREVIOUS PRESENTATION OR PUBLICATION DISCLOSURE

The results of the present study had not previously been presented at any scientific meeting and/or published in an abstract.

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