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An echocardiographic study of breed-specific reference ranges in healthy French Bulldogs

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Abstract

Echocardiography is a standard diagnostic tool for assessment of cardiac functions and cardiovascular diseases in dogs, however published echocardiographic measurements have varied widely based on dog breeds. The objective of this prospective reference interval study was to provide breed-specific echocardiographic values for healthy French Bulldogs. A total of 42 healthy French Bulldogs of both sexes (23 females and 19 males) were sampled. Furthermore, measurements for a control group (n = 16) were also conducted in four other dog breeds (Cocker Spaniel [n = 2], Cavalier King Charles Spaniel [n = 4], Terrier [n = 5], and Crossbreed [n = 5]). Standard M-mode, two-dimensional (2D), pulse wave (PW) Doppler, and tissue Doppler imaging (TDI) echocardiographic measurements were obtained from healthy French Bulldogs. The M-mode echocardiographic data obtained from French Bulldogs were compared to the data obtained from the control group. The left ventricular internal dimension at end-diastole (LVIDd; cm)/body surface area (BSA) (m^2) ratio for the study group was 3.35/0.53 = 6.32. Left ventricular measurements for French bulldogs and internal dimension at end-systole (21.23 ± 3.50 mm) and at end-diastole (33.50 ± 4.12 mm) were found to be significantly higher ($P < .001$) compared to control group values (left ventricular internal dimension at end-systole [LVIDs]; 17.46 ± 2.85 mm, LVIDd; 27.16 ± 4.20 mm, respectively). A significantly positive correlation in the French Bulldog group was noted between body weight and M-mode measurements (EPSS, IVSd, IVSs, LVIDd, LVIDs, and LVPWd). French bulldogs had a greater systolic and diastolic left ventricular volume than the control group. As a result, values reported in this study could be used as specific reference ranges in French Bulldogs.

KEYWORDS
dog, echocardiographic, pulsed wave Doppler, tissue Doppler imaging

1 | INTRODUCTION

Echocardiography is considered the most valuable diagnostic tool in the assessment of cardiac anatomy and functions as well as cardiovascular diseases in small animal practice.[1,2] Several researchers have investigated the echocardiographic reference values for the general dog population.[3,4,5,6] However, because of a large variations in body size and somatotype, reference ranges are very wide, and this

ABBREVIATIONS: 2D, two-dimensional; A, peak velocity of late transmitral flow; A' Sept, late diastolic motion wave recorded at the septal aspect of the mitral annulus; BSA, body surface area; E, peak velocity of early diastolic transmitral flow; E' Sept, early diastolic motion wave recorded at the septal aspect of the mitral annulus; E/A, ratio of E to A; LV, left ventricle; LVIDd, left ventricular internal dimension at end-diastole; LVIDs, left ventricular internal dimension at end-systole; MAPSE, mitral annular plane systolic excursion; M-Mode, motion mode; PW, pulsed wave; TDI, tissue Doppler imaging.

factor limits their clinical usefulness.[7,8] Reference values obtained from breed-specific echocardiographic studies have been reported to have a significant difference compared to the general population of healthy different dog breeds.[4,9,10,11] Therefore, breed-specific echocardiographic reference ranges may be more helpful in avoiding the misinterpretation of echocardiographic findings.[10,12] During an evaluation without considering the breed-specific echocardiographic reference values, a normal heart structure and/or measurement could end up being falsely interpreted as either large or small or increased or decreased when compared to the general nonbreed-specific reference ranges. These misinterpretations could lead to a misdiagnosis or an underdiagnosis of an underlying cardiac problem resulting in unnecessary/wrong treatment and/or no treatment.[10,12,13,14]

Studies investigating breed-specific echocardiographic values often employ two-dimensional (2D), M-mode, and pulse wave (PW) Doppler measurements.[10,14,15] However, few studies incorporated breed-specific tissue Doppler imaging (TDI) values.[16,17,18] Pulsed-wave TDI allows for real-time evaluation of myocardial motion velocity. Furthermore, this method is reported to be more sensitive than conventional imaging in the detection of early myocardial dysfunction that is due to cardiomyopathy and mitral insufficiency.[18,19,20]

There is not enough literature published on the normal echocardiographic reference ranges for French Bulldogs. The French bulldog is a small, brachycephalic breed. They have short and round bodies, with a large, deep, and full chest. We hypothesized that French Bulldogs are, therefore, likely to have cardiac structural and functional measurement differences than those of other breeds of similar size. In this study, our aim was to compare the left ventricle M-mode echocardiographic measurements of the French Bulldog breed with a control group (4 other dog breeds that are of similar size) and to develop breed-specific M-mode, 2D, PW Doppler, and TDI reference values for French Bulldogs. This study was also performed to investigate correlation of the echocardiographic measurements with age and body weight variables.

2 | METHODS

2.1 | Animals and study design

The study was a prospective, reference interval design. A total of 42 clinically healthy French Bulldogs of both sexes (23 females and 19 males) were recruited during the period of January to December in 2019 and evaluated at a single veterinary clinic (Truva Veterinary Clinic, Izmir, Turkey). Among these participants, a portion of the dogs (n = 12) came for their routine annual health checkup at this private clinic (Truva Veterinary Clinic, Izmir, Turkey). The other portion of the dogs (n = 30) were especially recruited for this study using the clinic vaccination record. The sample size was based on the patient vaccination records and the patients coming in for their annual health checkup and or wellness examination, hence considering these facts the research is based on convenience sampling.

M-mode echocardiographic data obtained from the French Bulldogs were compared to the data from a control group. The 2D, PW, and TDI Doppler measurements of French Bulldogs were not compared with those of the control group. M-mode measurements of the control group were collected retrospectively. There were 16 dogs in the control group, comprised of four other dog breeds (Cocker Spaniel [n = 2], Cavalier King Charles Spaniel [n = 4], Terrier [n = 5], and crossbreed [n = 5]). These control group breeds were chosen because they have body weights and sizes similar to those of French bulldogs. To evaluate the M-mode measurements of the control group dogs, breed-specific and general reference values were used.[6,21] Dogs in the control group were also clinically healthy based on physical examination, electrocardiographic (ECG), and echocardiographic study.

2.2 | Selection and description of subjects

This study was approved by the Erciyes University Animal Experiments Local Ethics Committee, and the approval certificate (Decision No: 19/121) was obtained. Furthermore, written informed consent authorizing study participation was provided from each dog owner.

Inclusion criteria were as follows: French Bulldog breed, aged between 1 and 10 years, male or female, healthy, fit condition, owned, with no history of cardiac disease, showing no signs of illness during physical, ECG, and echocardiographic examinations. Out of the 49 candidates, only 42 French bulldogs (7 French bulldogs were excluded) met the inclusion criteria for this study. The seven French Bulldogs were excluded from the study because of the following disqualifications. Cardiac auscultation revealed a holosystolic murmur in the mitral valve area in two dogs and the tricuspid valve area in one dog. Murmurs auscultated at the atrioventricular valve level were confirmed to be regurgitation by echocardiographic examination. Regurgitation peak velocity was measured as 2.98 in the dog with tricuspid insufficiency. The same dog was diagnosed with mild pulmonary hypertension. Increased amplitude of R wave in ECG and dilated cardiomyopathy in echocardiographic examination were detected in one dog. The echocardiographic evaluations were suboptimal for three dogs because of excessive movement.

No structural or functional anomalies were noted for each dog included in the present study in the standard M-mode, 2D, color Doppler, and PW Doppler echocardiographic examinations. Electrocardiograms revealed no conduction disturbances or ventricular/supraventricular arrhythmias in the study and control groups.

2.3 | Data recording and analysis

Standard bipolar (I, II, and III) and unipolar (aVR, aVL, and aVF) lead electrocardiograms (ECGs) were recorded (Biocare, ECG 300G, Guangdong, China) to detect cardiac arrhythmias and conduction disturbances. Echocardiographic studies were performed using a smart portable Doppler ultrasound device (Esaote, MyLab™Gamma, Genova, Italy) with multifrequency (1-4 MHz, 3-11 MHz) transducers. Following the physical examination, all dogs were shaved in the area on the

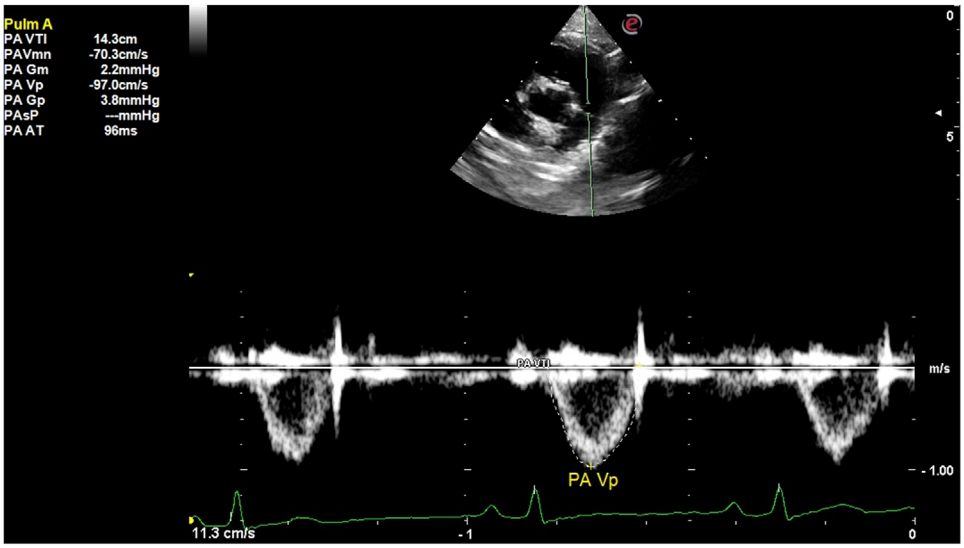

FIGURE 1 Pulsed wave (PW) Doppler image illustrating measurements acquired in the study group (French bulldog, n = 42). This echocardiographic sonogram was acquired from the right parasternal short axis level of pulmonary valve (1-5 MHz multi-frequency sector transducer). Sample volume was adjusted to as parallel with blood flow. The peak blood flow velocity was measured from the PW recordings as the maximal value of the systolic flow signal. Dashed lines (white) shows pulmonary artery velocity peak (PA Vp) [Color figure can be viewed at wileyonlinelibrary.com]

right and left chest wall, where the heartbeat was palpable (4th-6th intercostal space). Echocardiographic examinations were performed in right and left lateral recumbency in a dark and quiet room on a table with a cut-out. All dogs were unsedated during the examination procedure.

All decisions for subject inclusion/exclusion were made by two observers (V.G. and G.E.) with clinical experience (nearly 20 and 6 years, respectively) in small animal practice (diagnosis and treatment). All echocardiographic investigations were performed by only one operator (M.V., a veterinarian with 5 years of experience in echocardiography and registered cases [echocardiographic examination of 1500 different dogs]). The same observers (V.G. and G.E.) accompanied the operator (M.V.) during the data recording/selecting and analyses. The observers knew the following general information: breed, sex, health status, physical examination findings, vaccination information, castration status, and disease history. All those pre-requisites that were needed to select the dogs qualifying for the study. The decisions were based on consensus between the operator and the observers.

M-mode and 2D measurements were recorded according to the recommendations of American Society of Echocardiography and methodology published in the veterinary literature.[22,23]

Blood flow velocities in the pulmonary artery were recorded with pulsed wave (PW) Doppler from the right parasternal short axis view with the sample volume placed within the artery just distal to the pulmonary valve (Figure 1). The peak blood flow velocity was measured from the PW recordings as the maximal value of the systolic flow signal.

PW Doppler was employed to record aortic blood flow velocities. Measurements were taken from the left apical 5-chamber 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.

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 velocity.

A sample volume of 2-3 mm was set for all Doppler measurements. All Doppler time intervals and M-mode and 2D measurements were accompanied by a simultaneous 2-lead ECG recording.

In the TDI Doppler examination, high-frequency signals are eliminated because muscle tissue movement has a lower frequency than blood flow. Gain was minimized to obtain a clear tissue signal and minimum background noise. Sample volume was set at the mitral annulus. Peak myocardial velocities of systole (S'), early diastole (E'), and late diastole (A') were measured. Isovolumetric contraction time (IVCT) was measured from the end of the A' wave to the start of the S' wave. Isovolumetric relaxation time (IVRT) was measured from the end of the S' wave to the start of the E' wave (Figure 2). The index of myocardial performance (LIMP) was automatically calculated by the equipment following the above measurements.

2.4 | Statistics

The observer in the study team who selected and performed statistical analyses (G.E.) took a private course in applied statistics and data analysis equivalent to 96-h credits. The observer has also a PhD in veteri-

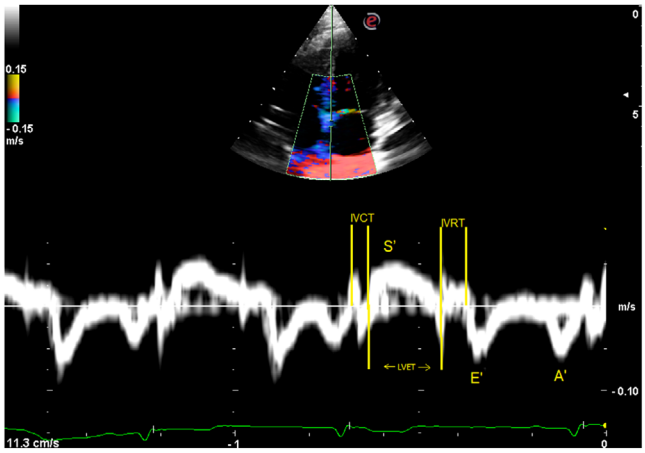

FIGURE 2 Tissue Doppler imaging (TDI) image illustrating measurements acquired from the septal mitral annulus in the study group (French Bulldog, n = 42). This echocardiographic sonogram was acquired from the left apical 4-chamber view (1-5 MHz multi-frequency sector transducer). The sample volume was placed in the mitral annulus. Abbreviations: A', late diastolic motion wave recorded at the septal aspect of the mitral annulus; E', early diastolic motion wave recorded at the septal aspect of the mitral annulus; IVCT, isovolumic contraction time; IVRT, isovolumic relaxation time; LVET, left ventricular ejection time; S', systolic tissue velocities [Color figure can be viewed at wileyonlinelibrary.com]

nary internal medicine. In addition, consultancy services for statistical analyses were also received from the Faculty of Veterinary Medicine, Department of Statistics. Statistical analyses were performed using commercially available software (SPSS for Windows Release 25.0 Program, SPSS Inc, Chicago, IL, USA). All data were graphically inspected and tested for normality using the Shapiro-Wilk test, and descriptive statistics were performed. The mean and SD are presented for all data that passed normality testing. Variables that failed normality testing are denoted with "*" and are presented as the median and interquartile range (IQR). The body weights (BWs) of the male and female dogs were compared with an independent samples *t*-test. The relationship between all variables and body weight and age was evaluated with Pearson and Spearman's rho correlation test. Simple linear regression analysis was evaluated on variables that were determined to have significant correlations ($P < .05$) with BW. An independent samples *t*-test was used to compare M-mode measurements of the French Bulldogs and control group dogs. A *P*-value of <.05 was considered to be significant.

Reference intervals were generated using another software program (NCSS Statistical software, NCSS LLC, Kaysville, Utah, USA). Reference intervals were computed using a robust method as recommended by the Clinical and Laboratory Standards Institute.[24,25] In this method, 3000 replications were performed for each echocardiographic variable. Reference intervals between 2.5% and 97.5% of the distribution were calculated, in addition to 90% confidence intervals of each limit.

3 | RESULTS

In the study group (French Bulldogs), the age ranged between 1 and 10 years (median, 4 years), and the mean BW was 12.03 ± 1.87 kg. A total of 54.8% of the dogs were female while 45.2% were male. There was no significant difference ($P = .801$) between male (12.11 ± 1.85 kg) and female dogs (11.96 ± 1.92 kg) in this study group (French Bulldogs) according to BWs. The average heart rate obtained during physical examination was 126 ± 9.84 beats/min in the study group. The left ventricular internal dimension at end-diastole (LVIDd; cm)/body surface area (BSA) (m^2) ratio for the study group was calculated and found to be 3.35/0.53 = 6.32.

For dogs in the control group (Cocker Spaniel [n = 2], Cavalier King Charles Spaniel

[n = 4], Terrier [n = 5], and crossbreed [n = 5]), the age ranged between 2 and 10 years (median, 6.5 years) and the mean BW was 10.5 ± 1.86 kg.

The M-mode, two-dimensional (2D), pulsed wave (PW), and tissue Doppler imaging (TDI) echocardiographic measurements obtained from the study group (French Bulldogs) are given in Tables 1-4, respectively.

For each variable (M-mode, 2D, PW Doppler, and TDI), 95% reference intervals (RIs) were established in the study group (French Bulldogs). In addition, the upper confidence limit of the upper end of the reference range and the lower confidence limit of the lower end of the reference range were calculated from 90% confidence intervals. For each limit, confidence intervals are provided in parentheses (Tables 1–4).

Significantly lower or greater M-mode parameters were detected in the study group compared to the control group (n:16, Cocker Spaniel, Cavalier King Charles Spaniel, Terrier, and crossbreed) and are given in Table 1. For instance, the LVIDd (33.50 ± 4.12 mm) and left ventricular internal dimension at end-systole (LVIDs; 21.23 ± 3.50 mm) values in the study group were found to be significantly higher than those in the control group (LVIDd [27.16 ± 4.20] and LVIDs [17.46 ± 2.85]). On the other hand, the LVPWd (6.99 ± 1.02 mm) value of the study group was significantly lower than the value (LVPWd (7.73 ± 1.39) obtained from the control group ($P < .05$). There were no other significant differences between the study group and the control group with respect to M-mode measurements.

Weak, significant, positive correlations were noted between body weight (BW) and the distance in space separating the anterior mitral valve leaflet from the septal wall, which is referred to as the E-point septal separation, or EPSS ($r = 0.325$, $P = .036$), the interventricular septal thickness at end-systole (IVSs; $r = 0.325$, $P = .036$), left ventricular internal dimension at end-systole (LVIDs; $r = 0.373$, $P = .015$), left ventricular posterior wall thickness at end-diastole (LVPWd; $r = 0.395$, $P = .010$) and left atrium diameter (LAD; mm; $r = 0.317$, $P = .041$) in the study group (French Bulldogs). A negative correlation between body weight and isovolumetric relaxation time (IVRT, ms; $r = -0.320$, $P = .039$) was also recorded in the study group (French bulldogs). Body weight and the interventricular septal thickness at the end-diastole (IVSd, mm; $r = 0.573$, $P < .001$), LVIDd (mm; $r = 0.514$, $P < .001$), left

TABLE 1 Comparison of M-mode echocardiographic measurements obtained from study group (French Bulldog, n = 42) and control group (4 other dog breeds, n = 16)

	Groups	Mean ± SD	95% RI *(90% CI of RI)*.95% PI (for control group) **	*P*-values
IVSd (mm)	Study group Control group	7.95 ± 0.998.08 ± 1.35	5.89 – 9.94*(5.50-6.35)* – *(9.48-10.38)*5.0 – 11.0**	.694
LVIDd (mm)	Study group Control group	33.50 ± 4.1227.16 ± 4.20	24.47 – 41.96*(22.92-26.23)* – *(39.53-43.69)*26.0 – 37.0**	**<.001**
LVPWd (mm)*	Study group Control group	6.70 (6.20-7.65)7.73 ± 1.39	4.64 – 8.97*(3.90-5.18)* – *(8.37-9.50)*5.0 – 10**	**.030**
IVSs (mm)	Study group Control group	11.18 ± 1.6911.11 ± 1.87	7.60 – 14.52*(6.73-8.52)* – *(13.62-15.43)*8.0 – 14.0**	.890
LVIDs (mm)	Study group Control group	21.23 ± 3.5017.46 ± 2.85	13.92 – 28.36(12.34-15.34) – (26.43-29.96)15.0 – 27.0**	**<.001**
LVPWs (mm)	Study group Control group	10.92 ± 1.4110.51 ± 1.19	7.99 – 13.80*(7.46-8.55)* – *(13.17-14.41)*8.0 – 15.0**	.315
FS (%)	Study group Control group	36.55 ± 5.5735.31 ± 4.17	24.44 – 47.16*(22.03-27.59)* – *(44.37-50.07)*25.0 – 40.0**	.425
EPSS (mm)	Study group	3.61 ± 0.75	2.07 – 5.16*(1.76-2.39)* – *(4.80-5.44)*	NC
EF (%)	Study group	67.14 ± 7.06	52.40 – 81.28*(49.50-56.14)* – *(78.19-84.35)*	NC
MAPSE (mm)	Study group	6.33 ± 0.42	5.48 – 7.20*(5.30-5.67)* – *(7.03-7.38)*	NC

Abbreviations: CI, confidence interval; EPSS, E-point to septal separation; EF, ejection fraction; FS, fractional shortening, IVSd, interventricular septal thickness at end-diastole; IVSs, interventricular septal thickness at end-systole; LVIDd, left ventricular internal dimension at end-diastole; 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; NC, not compared; PI, Prediction Interval. The mean and standard deviation (SD) are presented for all data which passed normality testing. Variables which failed normality testing are denoted with * and are presented as median and interquartile range (25th-75th percentile). The 95% reference interval (95% RI) was calculated using the robust method. For each parameter, the 95% RI is presented along with the 90% confidence interval for the 95% reference interval (90% CI of RI). ** 95% Prediction Interval (PI) for 11 kg body weight.[6] Bold text means that the p-value is significant.

TABLE 2 Left atrial dimension (LA) and aortic root diameter (Ao), 2D measurements obtained from study group (French Bulldog, n = 42)

	Mean ± SD	IQR	95% RI (90% CI of RI)
Ao dia (mm)	19.13 ± 1.38	18.08-20.0	16.26 – 21.92 (15.70-16.88) – (21.26-22.54)
LA dia (mm)*	20.0 (18.90- 22.45)	18.90- 22.45	12.40 – 28.34 (8.93-15.85) – (24.60-31.93)
LA/Ao*	1.06 (1.0-1.15)	1.0-1.15	0.85 – 1.29 (0.78-0.90) – (1.23-1.34)

Abbreviations: Ao, Aorta; LA, Left Atrium; LA/Ao, Left Atrium/Aorta, IQR, interquartile range. The mean and standard deviation (SD) are presented for all data which passed normality testing. Variables which failed normality testing are denoted with * and are presented as median and interquartile range (25th- 75th percentile). The 95% reference interval (95% RI) was calculated using the robust method. For each parameter, the 95% RI is presented along with the 90% confidence interval for the 95% reference interval (90% CI of RI).

ventricular posterior wall thickness at the end-systole (LVPWs; mm; $r = 0.437$, $P = .004$), aortic acceleration time (Ao AT; ms; $r = 0.494$, $P = .001$), and aortic (Ao) diameter (mm; $r = 0.471$, $P = .002$) were found to have moderate, significant, positive correlations in the study group (French Bulldogs). A significant but weak positive correlation between age and Ao AT ms ($r = 0.395$, $P = .010$) was observed in the study group (French Bulldogs). Negative correlations between age and mitral valve early diastolic flow acceleration time (MV EACC ms; $r = -0.317$, $P = .041$) and pulmonary artery velocity time integral (PAVTI m; $r = -0.354$, $P = .022$) were recorded in the study group (French Bulldogs). Age and early to late transmitral filling ratio (E'/A'; $r = -0.412$, $P = .007$) were found to be moderately and negatively correlated, and the correlation was considered significant in the study group (French Bulldogs). Significant linear regression with body weight was observed for EPSS (mm), IVSs (mm), IVSd (mm), LVIDs (mm), LVIDd (mm), LVPWs (mm), LVPWd (mm), Ao (mm), Ao AT (ms), and IVRT (ms) in the study group (French Bulldogs). Regression data with slopes and intercepts are presented in Table 5.

4 | DISCUSSION

In our study, echocardiographic reference ranges of healthy French Bulldogs were identified. The novel finding was that the French Bull-

TABLE 3 Pulsed wave (PW) Doppler measurements obtained from study group (French bulldog, n = 42)

	Median	IQR	95% RI (90% CI of RI)
Aortic VTI (m)	0.11	0.10-0.12	0.07 – 0.15 (0.06-0.08) – (0.13-0.16)
AV Vmax (m/s)	0.95	0.84-1.01	0.66 – 1.19 (0.61-0.72) – (1.14-1.25)
Ao AT (ms)	55.00	48.00-64.00	38.75 – 72.61 (36.49-41.04) – (67.64-77.42)
MV E (m/s)	0.76	0.69-0.83	0.59 – 0.93 (0.56-0.62) – (0.89-0.97)
MV A (m/s)	0.56	0.53-0.65	0.39 – 0.73 (0.36-0.42) – (0.68-0.78)
MV E/A	1.35	1.24-1.43	1.12 – 1.56 (1.08-1.17) – (1.52-1.60)
MV E DC (ms)	80.00	72.00-88.00	63.04 – 99.61 (60.34-66.08) – (94.76-103.47)
MV E ACC (ms)	48.00	48.00-64.00	31.78 – 73.04 (26.86-36.59) – (70.19-75.99)
PA VTI (m)	0.11	0.10-0.12	0.08 – 0.14 (0.076-0.088) – (0.13-0.15)
PA V mn	0.54	0.48-0.59	0.38 – 0.69 (0.35-0.42) – (0.66-0.72)
PA Gm (mmHg)	1.40	1.10-1.70	0.63 – 2.11 (0.46-0.78) – (1.94-2.27)
PA Vp (m/s)	0.86	0.77-0.95	0.63 – 1.09 (0.58-0.68) – (1.04-1.13)
PA Gp (mmHg)	2.95	2.40-3.60	1.39 – 4.55 (1.03-1.72) – (4.15-4.90)
PA AT (ms)	84.00	72.00-96.00	52.76 – 118.50 (45.29-58.19) – (108.07-126.59)

Abbreviations: Aortic VTI (m), aortic velocity-time integral; Ao AT (ms), Aortic acceleration time; AV Vmax (m/s), aortic valve peak velocity; MV E (m/s), mitral valve peak velocity of early diastolic transmitral flow; MV A (m/s), mitral valve peak velocity of late transmitral flow; MV E/A, mitral valve ratio of E to A; MV E DC (ms), mitral valve E velocity deceleration time; MV E ACC (ms), mitral walve early diastolic flow acceleration time; PA VTI (m), pulmonary artery velocity time integral; PA Vmax, pulmonary valve peak velocity; PA AT (ms), pulmonary artery velocity acceleration time, IQR, interquartile range. The median and interquartile range (25th- 75th percentile) are presented for all data. The 95% reference interval (95% RI) was calculated using the robust method. For each parameter, the 95% RI is presented along with the 90% confidence interval for the 95% reference interval (90% CI of RI).

TABLE 4 Tissue Doppler imaging (TDI) measurements obtained from study group (French Bulldog, n = 42)

	Median	IQR	95% RI (90% CI of RI)
E' sept (m/s)	0.08	0.07-0.09	0.06 – 0.10 (0.044-0.061) – (0.098-0.116)
A' sept (m/s)	0.06	0.05-0.07	0.03 – 0.09 (0.025-0.038) – (0.081-0.096)
E': A'	1.32	1.24-1.51	0.78 – 1.84 (0.66-0.92) – (1.71-2.00)
S' (m/s)	0.07	0.06-0.09	0.04 – 0.11 (0.035-0.049) – (0.099-0.115)
IVRT (ms)	40.0	32.00-40.00	24.00 – 50.00 (21.02-27.01) – (45.28-54.72)
IVCT (ms)	40.0	40.00-48.00	17.21 – 70.84 (10.88-24.10) – (60.27-75.21)
LVET	176.0	163.00-184.50	143.70 – 207.41 (135.99-151.50) – (200.84-214.76)
LIMP	0.48	0.42-0.58	0.31 – 0.67 (0.28-0.35) – (0.64-0.71)

Abbreviations: A' sept, late diastolic motion wave recorded at the septal aspect of the mitral annulus by pulsed-wave tissue doppler imaging; E' sept, early diastolic motion wave recorded at the septal aspect of the mitral annulus by pulsed-wave tissue doppler imaging; E'/A', early to late transmitral filling ratio; IVRT, isovolumic relaxation time; IVCT, isovolumic contraction time; LVET, left ventricular ejection time; LIMP, index of left ventricle myocardial performance; S', systolic tissue velocities, IQR, interquartile range. The median and interquartile range (25th-75th percentile) are presented for all data. The 95% reference interval (95% RI) was calculated using the robust method. For each parameter, the 95% RI is presented along with the 90% confidence interval for the 95% reference interval (90% CI of RI).

dogs were found to have a different M-mode measurement, especially the measurements of the left ventricle, compared to a control group of four other dog breeds.

The French Bulldog is a small, brachycephalic breed. They have short and round bodies, with a large, deep, and full chest.[26] Cardiac silhouette measured by vertebral heart score (VHS) has been reported to be greater in Bulldogs (French Bulldogs, English Bulldogs, and other Bulldogs) compared to that in some other breeds (Pug, Pomeranian, Yorkshire Terrier, Dachshund, Shih Tzu, Lhasa Apso, and Boston Terrier). It was reported that anomalous vertebrae in the thoracic column were associated with a significant increase in the VHS of the bulldog.[27] The larger LVIDd value of French Bulldogs may be one of the factors that causes a high VHS value. Left ventricular internal dimension at end-diastole and LVIDs measurements were recorded to establish the variation in left ventricle size in the study and control groups, and the average values of these parameters were found to be significantly higher in the study group (French Bulldogs) than in the control group ($P < .001$). Additionally, the LVIDd value obtained from

TABLE 5 Body weight correlation with echocardiographic parameters in the study group (French Bulldog, n = 42)

Parameters (units)	Regression (y)	Coefficient of determination (R^2)	Correlation (*r*)	*P*-value
EPSS (mm)	(0.131) x + 2.027	0.106	0,325	**.036**
IVSs (mm)	(0.295) x + 7.641	0.106	0.325	**.036**
IVSd (mm)	(0.303) x + 4.302	0.329	0.573	**<.001**
LVIDs (mm)	(0.698) x + 12.835	0.139	0.373	**.015**
LVIDd (mm)	(1.136) x + 19.840	0,264	0.514	**<.001**
LVPWs (mm)	(0.330) x + 6.949	0.191	0.437	**.004**
LVPWd (mm)	(0.230) x + 4.223	0.178	0.395	**.010**
LA (mm)	(0.515) x + 14.058	0.060	0,245	.118
Ao (mm)	(0.348) x + 14.948	0.222	0.471	**.002**
Ao AT (ms)	(2.380) x + 27.046	0.249	0.499	**.001**
IVRT (ms)	(−1.112) x + 52.423	0.107	0.327	**.034**

Abbreviations: EPSS, E-point to septal separation; IVSd, interventricular septal thickness at end-diastole; IVSs, interventricular septal thickness at end-systole; LVIDd, left ventricular internal dimension at end-diastole; 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; LA, Left Atrium, Ao, Aorta; Ao AT (ms), Aortic acceleration time; IVRT, isovolumic relaxation time. Linear regression analysis of selected echocardiographic variables with body weight using the equation y = ax + b, where y represents the echocardiographic variable, x represents body weight, a represents the slope, and b represents the intercept, R^2 values, correlation (r) and significance (P value) are provided for each variable. Bold text means that the p-value is significant.

the study group (French Bulldogs) in the present study was higher than the reported values for different breeds.[28,29,30,31] In our study, the LVIDd (cm)/body surface area (BSA) (m^2) ratios for French Bulldogs (3.35/0.53 = 6.32) were found to be higher than the values reported for other breeds in previous studies, such as Boxers (4.34), Doberman Pinschers (4.29), English Pointers (5.47), Golden Retrievers (4.46), Irish Wolfhounds (2.99), Afghan Hounds (5.19), and Great Danes (3.38).[2,28,29,30,31] The findings of this study suggest that French Bulldogs have a different cardiac M-mode measurement than other breeds.

Larger LVIDd values have been reported mainly in athletic breeds and training dogs.[5,32] Heart size is an important determinant of cardiac output, and therefore, aerobic capacity and exercise performance are affected in athletic breeds.[5,33,34] In contrast to athletic breeds, French Bulldogs have short and round bodies, but they have a large muscle mass. Therefore, it might also be a breed effect. In a similar study, a large LVIDd value in the Border Collie was explained by breed effects rather than athleticism.[10]

Healthy French bulldogs had higher LVIDs values ($P < .001$) and lower LVPWd values ($P = .030$) than the control group in the present study. Left ventricular internal dimension at end-systole is determined by the inotropic state of the myocardium and the degree of myofibril contraction affected by afterload.[10,35] The increase in LVIDs in French Bulldogs may be explained by the greater diastolic chamber size requiring minimum myocyte contraction to generate an adequate pulse at resting state.[11,35] An echocardiographic evaluation of French Bulldogs based on general or different dog breed population reference ranges may result in misdiagnosis (such as an early phase of dilated cardiomyopathy or heart failure) because this breed has a different chest structure, cardiac silhouette, and left ventricular internal dimensions compared to other breeds.[17,26,27]

Several researchers have reported echocardiographic reference values for different dog breeds.[10,12,13,14] To date, there has been a lack of data regarding echocardiographic reference values for French Bulldogs. Normal heart sizes and shapes show substantial variation based on dog breed. Breed-specific reference ranges should be taken into consideration when evaluating the heart.[36,37,38] Therefore, M-mode, 2D, PW, and TDI echocardiographic reference ranges for healthy French Bulldogs were defined in this study. Reference ranges reported in the present study may aid in diagnosing heart diseases in dogs of this breed. These values may also lay a foundation for future studies investigating the pathophysiology and the prevalence of heart diseases (congenital and acquired) among these dogs. Congenital heart diseases (such as ventricular septal defect and pulmonic stenosis) are seen in French Bulldogs rather than acquired heart diseases.[39,40] However, between 2014 and 2019, cases of dilated cardiomyopathy in this breed had been reported to the Food and Drug Administration (FDA)-Center for Veterinary Medicine (CVM),[41] and one case of dilated cardiomyopathy was also dropped from the present study.

In this study, significant positive correlations between body weight and systolic and diastolic M-mode measurements (EPSS, IVSd, IVSs, LVIDd, LVIDs, LVPWd, LA, and Ao) were noted in the study group. The positive correlation of different echocardiographic parameters with BW may be explained by the increase in heart size accompanying the increase in BW.[15,42] For some other breeds, positive correlations between left ventricle parameters and BW were also reported.[43]

Because of the strong linear relationship between BW and mitral annular plane systolic excursion (MAPSE), it is challenging to determine reference values for MAPSE in normal dogs. In a study aiming to identify MAPSE reference values based on body weight in different dog breeds, dogs weighing under 15 kg were found to have MAPSE val-

ues below 7 mm (6.5-7.5).[44] In agreement with this literature, we also found a mean MAPSE value of 6.35 ± 0.42 mm for the French Bulldog breed (6.0-6.63). A literature search revealed no MAPSE values for French Bulldog breeds to allow for comparison.

Tissue Doppler imaging measurements are commonly used in clinics as a marker of left ventricle systolic and diastolic dysfunction.[45,46,47] Human and canine studies report a decrease in the E':A' ratio with advancing age.[48,49,50] In humans, this condition is linked to a decline in diastolic functions of the heart as one grows older.[51] Similarly, we found a decrease in the E':A' ratio in conjunction with increasing age and a moderate negative correlation between age and the E':A' ratio. In addition, an E'/A' ratio of 1.32 (1.24-1.51) for healthy French Bulldogs was calculated in this study. These results were in line with the findings of previous studies.[52,53]

In the present study, E' sept and A' sept values in the study group were greater than the findings of Jihye Choi et al. (2013) concerning the same values.[53] However, when divided by BSA, the French Bulldog breed was found to have lower values. While the increases in body weight may account for the increased E' sept and A' sept values, division of these values by BSA resulting in lower values may be explained by breed differences. Furthermore, the E' sept measurement results in the present study were lower than the lateral TDI annulus measurement results reported in brachiocephalic dogs (French Bulldog, n = 22).[17] Similarly, there are some reports of septal mitral annulus measurements being lower than lateral mitral annulus measurements in both human and dog studies.[18,54,55,56] This condition may be explained by the fact that the ventricular septum is rooted in the right ventricle and heart base (aortic and pulmonary roots) and is not able to move around as much as the left ventricle free wall.[18]

Fractional shortening (FS) (%) (36.55 ± 5.57), E/A (1.35 (1.24-1.43), and E': A' (1.32 (1.24-1.51) values were measured in the study group to evaluate the left ventricular functions in the present study, and the findings were in line with measurements (FS [%]: 40.0 [36.7-45.0], E/A: 1.3 [1.2-1.4], E': A' [1.4 ± 0.2]) obtained from a study investigating cardiovascular changes in dogs with brachycephalic syndrome.[17]

4.1 | Limitations of the present study

Although physical and echocardiographic examination findings were within normal limits, the French Bulldogs in the present study could not be followed up. Therefore, potential subclinical disease may occur. Since follow-up examinations were not performed, this possibility cannot be ruled out. The sample sizes for the study group and the control group in the present study were quite low. In fact, data should ideally be analyzed from a minimum of 120 healthy individuals to establish an appropriate reference interval. However, if in some instances numbers between 40 and 120 are sampled, a robust methodology can be applied, which was the case in the present study. Furthermore, the study lacks the evaluation of intra- and interobserver variability. Another limitation of the study was that there were no sick dogs to confirm the robustness of the reference values obtained from our study.

5 | CONCLUSIONS

Healthy French Bulldogs were found to have a different cardiac M-mode measurement compared to the control group. Some echocardiographic parameters were found to be lower (LVPWd) or higher (LVIDd and LVIDs) than the values obtained from the control group. An echocardiographic examination that is not based on this breed-specific reference range may result in misdiagnosis. Values reported in this study may well be used as French bulldog breed-specific reference ranges.

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(c) Analysis and Interpretation of Data: Gunes, Ekinci, Vurucu

Category 2

(a) Drafting the Article: Vurucu, Ekinci
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Category 3

(a) Final Approval of the Completed Article: Gunes, Vurucu

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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