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Original article

Reference intervals for transthoracic echocardiographic measurements in adult Dachshunds

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Abstract

The aim of this study was to establish reference values for 2D and M-mode measurements in Dachshunds. Basic echocardiographic data, including M-mode, 2D and spectral Doppler measurements, was collected, analyzed and compared between 41 healthy Dachshunds and 50 other healthy dogs of similar weight. Echocardiographic reference intervals were prepared for Dachshunds. Dachshunds had a smaller left ventricular diameter in diastole and systole and a thicker septum than other dog breeds. Male Dachshunds had larger diastolic and systolic left ventricular diameter than females. Reference intervals for 2D and M-mode measurements in healthy Dachshunds differ from other dogs of similar weight and should be used for this breed to assess chamber enlargement.

Key words: echocardiography, Dachshund, dog, reference interval

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Introduction

Although generic reference values for echocardiographic measurements have been published for dogs (Crippa et al. 1992, Vollmar 1999, Cornell et al. 2004, Kayar et al. 2006, Muzzi et al. 2006, Bavegems et al. 2007, Cunningham et al. 2008, Boon 2011b, Castro et al. 2011, Jacobson et al. 2013, Gugjoo et al. 2014), recent years have shown that recorded values for specific dog breeds must be interpreted in light of their own normal values. The Dachshund is one of the more frequent small animal patients predisposed to heart disease, specifically chronic mitral valve disease (CMVD) (Kittleson 1998).The aim of this study was to compare basic echocardiographic measurements between Dachshunds and other breeds, and, if these proved significantly different, to establish separate reference intervals for echocardiographic measurements in Dachshunds.

Materials and Methods

The study complied with national and institutional guidelines regarding the use of animals in clinical research according to the Polish legal act of January 21st, 2005 (The law on Animal Experiments of January 21st, 2005), concerning experiments performed on client owned animals. All owners granted informed consent for inclusion of their pet's data in the study.

Initially medical history for each dog was reviewed and a full clinical examination was performed. Any chronic diseases, ongoing therapies and clinical signs revealed in the clinical examination were considered as exclusion criteria. Chronic illnesses, such as osteoarthritis were allowed as they did not affect the heart. Dogs were weighed using a standard veterinary scale and the body condition score (BCS) was recorded according to a widely accepted method (Laflamme 1997). The temperament was recorded for each dog and classed as normal or hyperactive. 41 Dachshunds, including 25 smooth haired , 9 long haired, and 7 wirehaired were included for determination of reference intervals (RI).

The echocardiographic examinations included two dimensional (2D), M-mode, colour Doppler and spectral Doppler assessment performed using ultrasound machines (Aloca 4000, Miro, Warsaw, Poland and Esaote MyLab Class C Vet, V.D. White Med, Warsaw, Poland) equipped with cardiology programs and 2.5-7.0 megahertz phased array transducers. All examinations were carried out without chemical restraint with the owners' present. The dogs were examined in right and left lateral recumbency on a specially constructed table with a cut-out for scanning (O'Grady et al. 1986, Thomas et al. 1993). In some cases, the examination was carried out on dogs in the standing position (Kittleson and Kienle 1998b, Vollmar 1999, Häggström 2005).

Measurements in the 2D views included the aortic root diameter (Ao) and left atrial diameter (LA), recorded from the right parasternal short axis view at the level of the heart base during diastole right after closure of the aortic valve. The aorta was measured along a line of the commissure of the noncoronary and right coronary valve cusps while the left atrium was measured along the commissure from the noncoronary and left coronary aortic valve line to the distal margin of the left atrium (Rishniw et al. 2000). Values for the E-point-to-septal separation (EPSS) were measured in M-mode views obtained from the right parasternal short axis view at the level of the mitral valve similarly to Kirberger (1992), but in the short axis view (Kittleson & Kienle 1998b). Values for the right and left ventricles, including the right ventricular diameter in diastole (RVDd), intervetricular septum in diastole (IVSd) and systole (IVSs), left ventricle diameter in diastole (LVDd) and systole (LVDs), and left ventricular free wall in diastole (LVWd) and systole (LVWs) were measured in M-mode views obtained from the right parasternal short axis views at the level of the papillary muscles. The left ventricular fractional shortening (FS) (Teichholtz method) and heart rate (HR) were also calculated from these M-mode views. The average of three consecutive measurements was recorded for all 2D and M-mode views. 2D and M-mode measurements were performed according to accepted methods (Sahn et al. 1978, Boon 2011a, Lang et al. 2015).

Colour Doppler studies were performed in all standard views for blood flow across each heart valve. Insignificant mitral or tricuspid regurgitation were accepted if valve leaflets were not changed, valvular prolapse was not present, and the regurgitant jet was of short duration, remained in close proximity to the valve leaflet and did not extend into the atrial chambers (Yuill et al. 1991, Jacobson et al. 2013). Spectral Doppler analysis was performed in the right parasternal short axis view at the level of the aorta and pulmonary artery for pulmonary artery flow (Pmax), and in the left five chamber view for aortic blood flow (Aomax) and for mitral inflow profile (E and A waves) and isovolumetric time (IVRT). The mitral inflow profile and IVRT were only measured in17 Dachshunds and 47 other dogs. The maximum flow velocities were recorded in all spectral Doppler pulsed wave measurements (Brown et al. 1991, Kirberger et al. 1992).

Statistical methods

Numerical variables (age, body weight, heart rate, echocardiographic measurements) were given as an arithmetic mean and standard deviation (SD) or a median and interquartile range (a distance between the lower quartile (Q1) and the upper (Q3) quartile; IQR) unless normally distributed according to a Shapiro-Wilk W test. The range was presented every time. Categorical variables were presented as the count and percentage and compared between groups using a Pearson's chi-square test. Descriptive statistics (Excel) were also used to show data for males and females separately.

To compare echocardiographic measurements between Dachshunds and other breeds weight-based echocardiographic ratio indices (wERIs) were calculated. The proportionality constant for the weight-based indices (k) was computed separately for Dachshunds (kd) and other dogs (ko) as a simple average of Aom/BW1/3: kd= 0.748±0.091 (0.565-0.912) for Dachshunds, ko=0.691±0.093 (0.495-0.956) for other breeds. A weight-based estimate of the aortic root dimension (Aow) was computed from the following equation: Aow = kd (for Dachshunds) or ko (for other dogs) × BW1/3. Weight-based echocardiographic ratio indices were obtained by dividing the corresponding raw M-mode measurements by Aow and they were denoted by a 'w' in front of their names, except for relative echocardiographic measurements not referenced to the individual body weight such as LA/Ao, fractional shortening (FS) and ejection fraction (EF). wERIs were compared between Dachshunds and other dogs by a Mann-Whitney U-test or an unpaired Student's t-test when normal distributions could be assumed. The relationship between body weight (BW) or heart rate (HR) and echocardiographic measurements was investigated using Pearson's linear correlation coefficient. Flow velocities and isovolumetric relaxation time were compared between Dachshunds and other dogs using an unpaired Student's t-test or analysis of covariance (ANCOVA) with HR as a covariant when a significant correlation with HR had been revealed. All statistical tests were two-tailed and a p-value of <0.05 was considered to indicate statistical significance.

To choose the best method for establishing reference intervals (RIs) echocardiographic measurements were analyzed first as raw (untransformed) data and then after a Box-Cox transformation: normality of distribution was assessed using a Shapiro-Wilk W test, asymmetry of distribution was assessed using Pearson's coefficient of skewness and outliers were identified with Horn's algorithm using Tukey's interquartile fence: an observation was considered as an outlier unless its value fell within a range from Q1 – 1.5×IQR to Q3+ 1.5×IQR. The set of data (untransformed or Box-Cox transformed) which appeared to approximate normality better (i.e. had an insignificant p-value, a coefficient of skewness closer to 0, and fewer outliers) was used for RIs determination. A robust method (Horn et al. 1998) was used every time due to the small sample size (i.e. <120) and a 90% confidence interval (90% CI) for a lower and upper reference limit was calculated using a bootstrap method (Horowitz et al. 2008). Statistical analyses were performed using a commercially available program (Statistica 12, StatSoft Inc., Krakow, Poland) except for RIs calculation, which was done using additional freeware in the Reference Value Adviser (Geffré et al. 2009).

Results

The study was carried out on 41 Dachshunds and 50 dogs of other breeds:18 mongrels, 5 each of Shetland Sheepdog and West Highland White Terrier, 3 Jack Russell Terriers, 2 each of Bedlington, Cairn, Norwich Terrier, Papillon and Miniature Schnauzer, and 1 of each Basenji, Cavalier King Charles Spaniel, Fox Terrier, Havanese, Miniature Pincher, Miniature Poodle, Pomeranian, Springer Spaniel, Welsh Terrier). There were 32 females among the Dachshunds (78%) and 37 females among the other breed dogs (74%). The dogs' age ranged from 9 months to 16 years with a median of 5.5 year and IQR between 2.4 and 9.9 years. BW ranged from 3.2 to 13.2 kg (7.05 to 29,1 lbs) with a median of 8.3 (18.3 lbs) and IQR between 6.0 and 10.0 kg (13.2 and 22.1 lbs). Neither age nor body weight differed significantly between the two groups (p=0.264 and p=0.631, respectively). Dachshunds had a significantly higher heart rate (HR) ranging from 82 to 183 beats per minute (bpm) with a median of 120 bpm and IQR from 110 to 147 bpm. In other breed dogs HR ranged from 60-154 bpm with a median of 104 bpm and IQR from 88-122 bpm (p<0.001). All dogs had a BCS of 4-6. Temperament was assessed in 35 Dachshunds and 50 dogs of other breeds, and a significantly higher percentage of Dachshunds were hyperactive – 16 (46%) vs. 7(14%), respectively, p=0.001.

Male and female Dachshunds were of the same age (p=0.218) and had comparable HR (p=0.133). Male Dachshunds were significantly heavier than females (median of 10.0 kg (22.1 lbs), IQR from 7.0 to 11.4 kg (15.4 to 25.1 lbs) and median of 7.7 kg (17.0 lbs) and IQR from 5.5 to 9.0 kg (12.1 to 19.8 lbs), respectively; p=0.049).

There were no macroscopic structural abnormalities of the mitral (including prolapse) or tricuspid valve leaflets seen in any of the dogs. However, traces of mi-

Table 1. A comparison of weight-based echocardiographic ratio indices (wERIs) for Dachshund and other dog breeds

	Dachshunds (n=41)		Other dogs (n=50)		p-value unpaired Student t-test or Mann-Whitney U test unless normally distributed
	Normality (Shapiro-Wilk test p-value)	Median, IQR, range	Normality (Shapiro-Wilk test p-value)	Median, IQR, range	
wAo	0.601	0.99, 0.93-1.10 (0.75-1.22)	0.172	1.00, 0.91-1.07 (0.72-1.38)	0.999
wLA	0.310	1.27, 1.11-1.45 (0.93-1.72)	0.568	1.22, 1.10-1.30 (0.89-1.58)	0.067
LA / Ao	0.239	1.28, 1.18-1.35 (0.92-1.74)	0.678	1.21, 1.11-1.32 (0.75-1.54)	0.051
wRVDd[b]	0.966	0.46, 0.41-0.53 (0.24-0.69)	<0.001[a]	0.61, 0.50-0.72 (0.27-1.48)	<0.001*
wIVSd	<0.001[a]	0.46, 0.42-0.51 (0.34-0.83)	0.006[a]	0.42, 0.40-0.48 (0.31-0.70)	0.047*
wIVSs	0.168	0.66, 0.58-0.77 (0.49-0.99)	0.081	0.64, 0.59-0.69 (0.46-0.98)	0.155
wLVDd	0.480	1.73, 1.60-1.89 (1.31-2.06)	0.093	1.90, 1.80-2.03 (1.51-2.45)	<0.001*
wLVDs	0.091	1.09, 0.83-1.19 (0.56-1.37)	0.037[a]	1.18, 1.11-1.29 (0.89-1.69)	<0.001*
wLVWd	<0.001[a]	0.44, 0.38-0.49 (0.31-0.86)	0.372	0.43, 0.39-0.48 (0.34-0.58)	0.883
wLVWs	<0.001[a]	0.64, 0.62-0.76 (0.50-1.13)	0.595	0.64, 0.58-0.69 (0.45-0.85)	0.340
wEPSS	0.192	0.12, 0.09-0.14 (0.00-0.25)	<0.001[a]	0.11, 0.08-0.14 (0.04-0.40)	0.851
FS%	0.046[a]	39, 36-47 (28-67)	0.145	38, 36-40 (29-48)	0.053

[a] non-normal distribution of the measurement; [b] 34 Dachshunds included; * statistically significant difference between Dachshunds and other dogs; IQR interquartile range

tral regurgitation were noted in two Dachshunds (2/41, 4.8%) and 4 other dogs (4/50, 8.0%), tricuspid regurgitation in 3 Dachshunds (3/41, 7.3%) and 5 other dogs (5/50, 10%), and pulmonary valve insufficiency in one Dachshund (1/41, 2.4%) and 2 other dogs (2/50, 4%). No valve or pulmonary artery abnormalities were noted and no aortic valve insufficiency was recorded. The RVDd was recorded for 34 out of the 41 Dachshunds, and in the remaining dogs a precise measurement was unattainable. Only 17 of the Dachshunds had mitral inflow and outflow Doppler measurements recorded.

Controlling for body weight, the Dachshunds proved to have significantly smaller diameter of the right and left ventricle in diastole and of the left ventricle in systole, as well as significantly thicker interventricular septum in diastole than dogs of other breeds (Table 1). Among the Dachshunds, males had a significantly larger left ventricular diameter in diastole and systole, and lower FS compared to females (Table 2). Table 3 presents descriptive statistics of 2D and M-mode measurements for all dogs and male and female dogs separately.

Maximal aortic flow (Amax), as well as E and A wave velocities were significantly lower in Dachshunds compared to other dog breeds (Table 4).

There was a positive correlation between body weight and some echocardiographic measurements both in Dachshunds (Ao, LA, IVSd, IVSs, LVDd, LVDs, LVWs) and in all dogs (the seven aforementioned plus LVWd and EPSS) (Table 5).

Table 6 presents raw echocardiographic data as well as descriptive statistics and reference intervals of 2D and M-mode echocardiographic measurements for healthy Dachshunds.

Discussion

In recent years many breed specific echocardiographic parameters have been published for canines (Crippa et al. 1992, Morrison et al. 1992, Vollmar 1999,

Table 2. Differences between male and female weight-based echocardiographic indices (wERIs) in Dachshunds.

Weight-based echocardiographic ratio indices (wERIs)	Median, IQR, range		p-value unpaired Student's t-test or Mann-Whitney U test unless normally distributed
	Females (n=32)	Males (n=9)	
wAo	1.02, 0.93-1.12 (0.76-1.22)	0.94, 0.89-1.00 (0.83-1.13)	0.277
wLA	1.25, 1.10-1.44 (0.93-1.72)	1.31, 1.17-1.48 (1.05-1.55)	0.623
LA / Ao	1.27, 1.15-1.34 (0.92-1.74)	1.32, 1.28-1.39 (1.17-1.70)	0.157
wRVDd[a]	0.49, 0.42-0.53 (0.24-0.69)	0.43, 0.41-0.52 (0.31-0.58)	0.356
wIVSd[b]	0.46, 0.43-0.53 (0.35-0.83)	0.44, 0.41-0.48 (0.39-0.54)	0.278
wIVSs	0.67, 0.62-0.79 (0.49-0.99)	0.59, 0.56-0.73 (0.53-0.84)	0.251
wLVDd	1.68, 1.54-1.84 (1.31-2.06)	1.93, 1.82-1.98 (1.67-2.06)	0.004
wLVDs	0.98, 0.81-1.12 (0.56-1.37)	1.23, 1.14-1.28 (1.08-1.31)	0.001
wLVWd[b]	0.45, 0.39-0.50 (0.31-0.86)	0.41, 0.37-0.49 (0.33-0.53)	0.378
wLVWs[b]	0.65, 0.62-0.77 (0.5-1.13)	0.64, 0.56-0.68 (0.53-0.80)	0.369
wEPSS	0.1, 0.08-0.14 (0-0.25)	0.12, 0.11-0.13 (0.07-0.19)	0.418
FS%[b]	0.43, 0.36-0.49 (0.29-0.67)	0.37, 0.37-0.38 (0.28-0.43)	0.039

[a] 34 Dachshunds included; [b] non-normal distribution of the measurement; IQR interquartile range

O'Leary et al. 2003, Guglielmini et al. 2006, Kayar et al. 2006, Muzzi et al. 2006, Bavagems et al. 2007, Cunningham et al. 2009, Vörös et al. 2009, Castro et al. 2011, Jacobson et al. 2013, Gugjoo et al. 2014) showing significant differences between these measurements in different dog breeds (Boon 2011b). This underlines the necessity to prepare reference values for specific breeds, especially ones having different chest conformations (Morrison et al. 1992, Della Torre et al. 2000, Boon 2011b) or athletic ability (Jacobson et al. 2013). During the preparation of this study echocardiographic parameters for Dachshunds were published (Lim et at. 2016). Similarities between our results and those presented by Lim et al. (2016) include measurements of the aortic diameter, LVDd, LVDs, LVWd, LVWs, EPSS and FS; however, our study showed a slightly smaller LA diameter and LA/Ao ratio, and a slightly thicker IVSd and IVSs.

HR may be related to BW, with smaller animals having a faster HR, and age, with younger animals having a faster heart rate (Hezzell 2013). The Dachshunds were neither younger nor lighter than the group of other dogs, however they appeared more hyperactive than other dogs. Although temperament is a somewhat subjective assessment, this is a plausible reason for the difference in HR.

Published studies to date have yielded inconsistent results regarding the relationship between gender and echocardiographic measurements. Some have shown no link (O'Leary et al. 2003, Vörös et al. 2009, Gugjoo et al. 2014), whereas others have revealed some influence of gender on echocardiographic parameters, including the Beagle (Crippa et al. 1992), German Shepherd (Kayar et al. 2006), and Whippet (Bavagems et al. 2007). In our studies the Dachshund males were significantly heavier than females, but only minimally, which might explain the minimally larger left ventricle in systole and diastole in males (Table 2 and 3). However, to overcome a possible bias resulting from a different body weight, wERIs were compared between breeds. Of interest is the fact that once again a much smaller number of unaffected males compared to females is reported with respect to heart disease (Garncarz et al. 2013). It remains a challenge to find male Dachshund dogs without relevant echocardiographic changes.

Table 3. Descriptive statistics for male and female 2D and M-mode measurements in Dachshunds.

	Females (n=32)			Males (n=9)		
	Mean (SD)	Median (IQR)	Range	Mean (SD)	Median (IQR)	Range
LA	1.83 (0.26)	1.80 (1.63-1.99)	1.40-2.37	2.04 (0.39)	2.16 (1.7-2.22)	1.48-2.62
Ao	1.47 (0.18)	1.48 (1.35-1.56)	1.10-1.80	1.50 (0.25)	1.55 (1.32-1.6)	1.16-1.99
LA / Ao	1.26 (0.17)	1.27 (1.15-1.34)	0.92-1.74	1.35 (0.15)	1.32 (1.28-1.39)	1.17-1.70
RVDd	0.71 (0.17)	0.70 (0.57-0.8)	0.37-1.08	0.68 (0.15)	0.70 (0.56-0.75)	0.50-0.93
IVSd	0.70 (0.14)	0.70 (0.6-0.8)	0.45-1.09	0.70 (0.14)	0.68 (0.64-0.80)	0.52-0.96
IVSs	1.01 (0.20)	1.00 (0.84-1.15)	0.64-1.41	1.01 (0.26)	0.93 (0.80-1.20)	0.73-1.49
LVDd	2.46 (0.32)	2.50 (2.16-2.7)	1.76-3.00	2.97 (0.42)	2.94 (2.66-3.3)	2.47-3.47
LVDs	1.41 (0.31)	1.44 (1.27-1.59)	0.72-2.10	1.89 (0.23)	1.91 (1.68-2.1)	1.55-2.21
LVWd	0.67 (0.15)	0.64 (0.59-0.75)	0.39-1.14	0.67 (0.15)	0.70 (0.59-0.76)	0.43-0.86
LVWs	1.01 (0.20)	1.00 (0.89-1.18)	0.64-1.49	1.01 (0.19)	1.08 (0.9-1.1)	0.69-1.28
EPSS	0.16 (0.07)	0.16 (0.12-0.2)	0.00-0.40	0.20 (0.06)	0.20 (0.19-0.21)	0.09-0.30
FS%	43 (8)	43 (36-49)	29-67	36 (4)	37 (37-38)	28-43
EF%	75 (11)	75 (68-82)	42-95	75 (11)	75 (68-82)	42-95

SD standard deviation, IQR interquartile range

Table 4. Comparison of flow velocities and isovolumetric relaxation time between Dachshunds and other dogs.

	Dachshunds		Others		An unpaired Student's t-test or ANCOVA([a])
	n	Mean±SD (range)	n	Mean±SD (range)	
E wave	17	0.64±0.16 (0.42-0.94)	46	0.74±0.16 (0.47-1.20)	0.029
A wave	17	0.60±0.12 (0.44-0.92)	46	0.70±0.16 (0.33-1.11)	0.039
E/A	17	1.08±0.27 (0.66-1.48)	46	1.12±0.32 (0.54-2.02)	0.719
IVRT	17	61.2±9.2 (37-78)	46	68.3±12.6 (46-108)	0.146[a]
Amax	41	1.12±0.22 (0.66-1.57)	50	1.24±0.25 (0.73-1.70)	0.018
Pmax	41	0.95±0.28 (0.42-1.71)	50	0.96±0.22 (0.56-1.52)	0.078[a]

[a] adjusted by HR which is significantly correlated with IVRT and Pmax and also as differs between Dachshunds and other breeds, SD standard deviation

Although all flow velocities in the Dachshund and other dog breeds were within previously reported reference intervals (Boon 2011b), the aortic flow, E wave and A wave velocities were slightly slower in Dachshunds (Table 4). Flow velocities are partly dependent on the diameter of the blood vessel (Kittleson and Kienle 1998b). However, the diameter of the aorta did not differ between the two groups, so this does not explain the different velocities. One study has shown a significant effect of HR and heart mass on blood flow velocities, with lower mass and higher HR associated with increased velocities (Kirberger et al. 1992). The mass in this study was not evaluated; however, the HR in Dachshunds was higher than in the other dog

Table 5. Correlation of body weight (BW) with echocardiographic values measured in Dachshunds and other dog breeds and only Dachshunds.

Echocardiographic measurements	BW [kg] of all 91 dogs		BW [kg] of 41 Dachshunds	
	Pearson linear correlation coefficient	p-value	Pearson linear correlation coefficient r	p-value
Ao	0.56	<0.001	0.47	0.001
LA	0.50	<0.001	0.41	0.007
LA / Ao	0.003	0.976	0.05	0.772
RVDd	0.21[a]	0.053	0.21	0.240
IVSd	0.43	<0.001	0.49	0.001
IVSs	0.42	<0.001	0.43	0.005
LVDd	0.73	<0.001	0.69	<0.001
LVDs	0.56	<0.001	0.50	0.001
LVWd	0.37	<0.001	0.29	0.067[b]
LVWs	0.40	<0.001	0.33	0.038
EPSS	0.34	0.001	0.12	0.450

[a] 84 dogs examined, of which 34 were Dachshunds
[b] lack of correlation results from one outlying value (LVWd=1.14); without this value r=0.45, p=0.004

Table 6. Raw data and reference intervals of 2-D and M-mode echocardiographic measurements established for healthy adult Dachshunds.

Echocardiographic measurements	Descriptive statistics										Reference interval (RI)[a]	
	Raw data							Box-Cox transformed data				
	n	Mean (SD)	Median	Range	Normality p-value	Coefficient of skewness	Tukey's outliers	Normality p-value	Coefficient of skewness	Tukey's outliers	Lower limit (90% CI)	Upper limit (CI 90%)
Ao	41	1.47 (0.19)	1.49	1.10-1.99	0.619	0.392	1	0.894	0.0003	1	1.12 (1.06, 1.19)	1.91 (1.80, 2.02)
LA	41	1.88 (0.30)	1.83	1.40-2.62	0.100	0.536	1	0.465	0.045	0	1.37 (1.30, 1.44)	2.63 (2.43, 2.84)
LA / Ao	41	1.28 (0.17)	1.28	0.92-1.74	0.239	0.464	2	0.523	0.002	3	0.96 (0.90, 1.04)	1.65 (1.56, 1.74)
RVDd	34	0.70 (0.16)	0.70	0.37-1.08	0.732	0.193	0	0.778	-0.021	0	0.39 (0.33, 0.46)	1.05 (0.97, 1.15)
IVSd	41	0.70 (0.14)	0.69	0.45-1.09	0.412	0.549	0	0.965	0.004	0	0.46 (0.42, 0.50)	1.03 (0.94, 1.12)
IVSs	41	1.01 (0.21)	1.00	0.64-1.49	0.158	0.426	0	0.458	0.013	0	0.64 (0.59, 0.70)	1.50 (1.38, 1.63)
LVDd	41	2.57 (0.40)	2.60	1.76-3.47	0.194	0.414	2	0.444	0.0001	0	1.85 (1.74, 2.00)	3.48 (3.25, 3.71)
LVDs	41	1.51 (0.36)	1.51	0.72-2.21	0.813	-0.073	0	0.815	-0.052	0	0.78 (0.65, 0.95)	2.25 (2.09, 2.40)
LVWd	41	0.67 (0.15)	0.65	0.39-1.14	0.092	0.869	1	0.891	-0.003	2	0.42 (0.38, 0.47)	1.01 (0.91, 1.13)
LVWs	41	1.01 (0.19)	1.00	0.64-1.49	0.798	0.337	0	0.978	-0.007	0	0.66 (0.60, 0.72)	1.45 (1.34, 1.56)
EPSS	41	0.17 (0.07)	0.18	0.00-0.40	0.003	0.339	1	0.025	0.00003	1	0.03 (0.00, 0.07)	0.31 (0.27, 0.36)
FS%	41	0.41 (0.08)	0.39	0.28-0.67	0.046	0.902	1	0.873	0.008	0	0.29 (0.28, 0.31)	0.61 (0.55, 0.67)

SD standard deviation

breeds, yet flow velocities were lower. Previous reports have demonstrated slightly different aortic flow velocities (O'Leary et al. 2003, Muzzi et al. 2006, Bavagems et al. 2007, Jacobson et al. 2013) as well as E and A wave velocities (Muzzi et al. 2006, Bavagems et al. 2007, Jacobson et al. 2013) in different dog breeds. In light of this and the fact that the reported values remain within previously reported reference ranges, these differences may be considered clinically irrelevant. However, further studies would be needed to elucidate the reason for this difference.

The positive correlation seen in both groups of dogs assessed together (Dachshunds and other dogs) between BW and Ao, LA, IVSd, IVSs, LVDd, LVDs, and LVWs is quite logical and in line with other publications (Cornell 2004, Bavagems 2007, Cunningham 2008, Geffre 2009, Boon 2011b). Simply put, the larger the animal, the larger the heart. The LVWd was correlated to BW when both groups of dogs were analyzed for correlation with BW, but not when the correlation was assessed in the Dachshunds alone with BW, due to one Dachshund with an outlying LVWd value. There should be no influence of BW on indexed values or maximal flow velocities, therefore the lack of correlation is expected and has previously been shown (Cunningham et al. 2008). EPSS was also correlated positively with BW when all dogs were analyzed, but not in Dachshunds alone. The latter is most likely a result of the small group of dogs, as an increase in this parameter is usually seen in larger dogs (Boon 2011a).

According to Brown and colleagues (2003) the species-dependent proportionality constant for the weight-based indices (kdog) is 0.795. However, in our study k was significantly lower for kdog (mean difference of 0.078, 95% CI: 0.058, 0.098, $p<0.001$), both for Dachshunds (kd) (mean difference of 0.047, 95% CI: 0.018, 0.075, $p=0.002$) and for other breeds (ko) (mean difference of 0.104, 95% CI: 0.077, 0.130, $p<0.001$). k was also different between Dachshunds and other breeds ($p<0.001$). Therefore, we used k calculated separately for Dachshunds and other breeds.

In this study weight adjusted parameters that differed significantly between the Dachshunds and other dog breeds included wRVDd, wLVDd, wLVDs and wIVSd. Others have reported the left ventricle as larger in dogs with increased athletic abilities (Stepien 1998). This does not imply necessarily athletic dogs, but rather breeds bred for athletic abilities such as the Border Collie (Jacobson et al. 2013) or Greyhounds (Snyder 1995). However, the right and left ventricular lumen in diastole and left ventricular lumen in systole were significantly smaller than in the other dog breeds, while the IVSd was minimally but significantly thicker. It appears that the different size heart in the Dachshunds could simply be a characteristic of the breed, possibly resulting from a smaller body size compared to other dog breeds, a different chest conformation, or even the influence of systemic blood pressure. The remaining two-dimensional and M-mode weight-adjusted echocardiographic measurements did not differ significantly between the two groups of dogs.

Based on this study a left ventricular lumen size normal for other breeds may be considered slightly enlarged for the Dachshund. To minimize the chance of erroneously accepting an enlarged left ventricle as normal, Dachshund specific values should be used. This is especially important when classifying disease stage in Dachshunds with chronic mitral valve disease according to the most recent guidelines (Atkins et al. 2009) as this disease readily leads to chamber enlargement, including the left atrium and left ventricle.

The main drawback to this study is the small sample size. Forty one dogs is the number which allows for RIs calculation when special statistical methods are applied (Geffre et al. 2011), but does not allow for any further partitioning (e.g. into males and females). Also, there was a larger group of females than males, which is a result of males being affected more often with subclinical disease at a younger age. In other words, there simply were no more unaffected males found during the study period and this has been seen in previous publications (Garncarz et al). Another drawback is the high weight variability of the Dachshunds, and it appears that other studies have faced similar difficulties (Kayar et al. 2006, Bavegems et al. 2007, Gugjoo et at. 2014, Misbach et al. 2014).

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References

Atkins C, Bonagura J, Ettinger S, Fox P, Gordon S, Haggstrom J, Hamlin R, Keene B, Luis-Fuentes V, Stepien R

(**2009**) Guidelines for the Diagnosis and Treatment of Canine Chronic Valvular Heart Disease. J Vet Intern Med 23: 1142-1150.

Bavegems V, Duchateau L, Sys SU, De Rick A (**2007**) Echocardiographic reference values in whippets. Vet Radiol Ultrasound 48: 230-238.

Boon JA (**2011a**) The two-dimensional echocardiographic exam. In: Boon JA (ed) Veterinary Echocardiography. 2nd ed., Wiley-Blackwell, Oxford, pp 71-72.

Boon JA (**2011b**) Appendix Two Canine. In: Boon JA (ed) Veterinary Echocardiography. 2nd ed., Wiley-Blackwell, Oxford. pp 531-558.

Brown DJ, Knight DH, King RR (**1991**) Use of pulsed-wave Doppler echocardiography to determine aortic and pulmonary velocity and flow variables in clinically normal dogs. Am J Vet Res 52: 543-550.

Brown DJ, Rush JE, MacGregor J, Ross JN Jr, Brewer B, Rand WM (**2003**) M-mode echocardiographic ratio indices in normal dogs, cats, and horses: a novel quantitative method. J Vet Intern Med 17: 653–662.

Castro MG, Torres RC, Araujo RB, Muzzi RA, Silva EF (**2011**) Echocardiography of clinically normal Yorkshire Terrier dogs. Arq Bras Med Vet Zootec 63: 1079-1086.

Cornell CC, Kittleson MD, Della Torre P, Haggstrom J, Lombard CW, Pedersen HD, Vollmar A, Wey A (**2004**) Allometric Scaling of M-Mode Cardiac Measurements in Normal Adult Dogs. J Vet Intern Med 18: 311-321.

Crippa L, Ferro E, Melloni E, Brambilla P, Cavalletti E (**1992**) Echocardiographic parameters and indices in the normal beagle dog. Lab Anim 26: 190-195.

Cunningham SM, Rush JE, Freeman LM, Brown DJ, Smith CE (**2008**) Echocardiographic Ratio Indices in Overtly Healthy Boxer Dogs Screened for Heart Disease. J Vet Intern Med 22: 924-930.

Della Torre PK, Kirby AC, Church DB, Malik R (**2000**) Echocardiographic measurements in greyhounds, whippets and Italian greyhounds -- dogs with a similar conformation but different size. Aust Vet J. 78: 49-55.

Garncarz M, Parzeniecka-Jaworska M, Jank M,Łój M (**2013**) Retrospective study of clinical signs and epidemiology of valve disease in a group of 207 Dachshunds in Poland. Acta Vet Scand 55: 52.

Geffré A, Braun JP, Trumel C, Concordet D (**2009**) Estimation of reference intervals from small samples: an example using canine plasma creatinine. Vet Clin Pathol 38: 477-484.

Geffré A, Concordet D, Braun JP, Trumel C (**2011**) Reference Value Advisor: a new freeware set of macroinstructions to calculate reference intervals with Microsoft Excel. Vet Clin Pathol 40: 107–112.

Gugjoo MB, Hoque M, Saxena AC, Shamsuz Zama MM, Dey S (**2014**) Reference values of M-mode echocardiographic parameters and indices in conscious Labrador Retriever dogs. Iran J Vet Res 15: 341-346.

Guglielmini C, Rocconi F, Brugnola L, Valerio F, Mattei L, Boari A (**2006**) Echocardiographic and Doppler echocardiographic findings in 11 wolves (Canis lupus). Vet Rec 158: 125-129.

Häggström J, Kvart C, Pedersen HD (**2005**) Acquired valvular disease. In: Ettinger S, Feldman EC (eds) Textbook of Veterinary Internal Medicine. 6th ed., Elsevier Saunders, St. Louis, Missouri, pp 1022-1039.

Hezzel MJ, Humm K, Dennis SG, Agee L, Boswood A (**2000**) Relationships between heart rate and age, bodyweight and breed in 10,849 dogs. J Small Anim Pract 54: 318-324.

Horn PS, Pesce AJ, Copeland BE (**1998**) A robust approach to reference interval estimation and evaluation. Clin Chem 44: 622-631.

Horowitz GL, Altaie S, Boyd J, Ceriotti FC, Garg U, Horn P, Pesce A, Sine HE, Zakowski J (**2008**) Clinical and Laboratory Standards Institute (CLSI). Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guidelines. 3rd ed. CLSI (Clinical and Laboratory Standards Institute) document C28-A3. Wayne, PA: Clinical and Laboratory Standards Institute: 3.

Jacobson JH, Boon JA, Bright JM (**2013**) An echocardiographic study of healthy Border Collies with normal reference ranges for the breed. J Vet Cardiol 15: 123-130.

Kayar A, Gonui R, Or ME, Uysal A (**2006**) M-mode echocardiographic parameters and indices in the normal German shepherd dog. Vet Radiol Ultrasound 47: 482-486.

Kirberger RM, Bland-Van Den Berg P, Darazs B (**1992**) Doppler echocardiography in the normal dog: Part II. Factors affecting blood flow velocities and a comparison between left and right heart flow. Vet Radiol Ultrasound 33: 380-386.

Kittleson MD (**1998**) Myxomatous Atrioventricular Valvular Degeneration. In: Kittleson MD, Kienle RD (eds) Small Animal Cardiovascular Medicine. Mosby Inc, St Louis, pp 297-317.

Kittleson MD, Kienle RD (**1998a**) Normal Clinical Cardiovascular Physiology. In Kittleson MD, Kienle RD (eds) Small Animal Cardiovascular Medicine. Mosby Inc, St Louis, pp 11-15.

Kittleson MD, Kienle RD (**1998b**) Echocardiography. In: Kittleson MD. Kienle RD (eds) Small Animal Cardiovascular Medicine. Mosby Inc, St Louis, pp 95-117.

Laflamme DP (**1997**) Development and validation of a body condition score system for dogs. Canine Practice 22: 10-15.

Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, Flachskampf FA, Foster E, Goldstein SA, Kuznetsova T, Lancellotti P, Muraru D, Picard MH, Rietzchel ER, Rudski L, Spencer KT, Tsang W, Voigt JU (**2015**) Recommendations for Cardiac Chamber Quantification by Echocardiography in Adults: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr 28: 1-39.

Lim CK, Fosgate GT, Green HW 3rd, Kirberger RM (**2016**) Two-dimensional left atrium-to-aorta ratios and left ventricular M-mode transthoracic echocardiographic measurements in clinically normal adult Dachshunds. Am J Vet Res 77: 374-382.

Misbach C, Lefebvre HP, Concordet D, Gouni V, Trehiou-Sechi E, Petit AM, Damoiseaux C, Leverrier A, Pouchelon JL, Chetboul V (**2014**) Echocardiography and conventional Doppler examination in clinically healthy adult Cavalier King Charles Spaniels: effect of body weight, age, and gender and establishment of reference intervals. J Vet Cardiol 16: 91-100.

Morrison SA, Moise NS, Scarlett J, Mohammed H, Yeager AE (**1992**) Effect of breed and body weight on echocardiographic values in four breeds of dogs of different somatotype. J Vet Intern Med 6: 220-224.

Muzzi RA, Muzzi LA, de Araujo RB, Cherem M (**2006**) Echocardiographic indices in normal German shepherd dogs. J Vet Sci 7: 193-198.
O'Grady MR, Bonagura JD, Powers JD, Herring DS (**1986**) Quantitative cross sectional echocardiography in the normal dog. Vet Radiol Ultrasound 27: 34-49.
O'Leary CA, Mackay BM, Taplin RH, Atwell RB (**2003**) Echocardiographic parameters in 14 healthy English Bull Terriers. Aust Vet J 81: 535-542.
Rishniw M, Hollis NE (**2000**) Evaluation of Four 2-Dimensional Echocardiographic Methods of Assessing Left Atrial Size in Dogs. J Vet Intern Med 14: 429-435.
Sahn DJ, DeMaria A, Kisslo J, Weyman A (**1978**) Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. Circulation 58: 1072-1083.
Snyder PS, Sato T, Atkins CE (**1995**) A comparison of echocardiographic indices of the non-racing healthy greyhound to reference values from other breeds. Vet Rardiol Ultrasound 36: 387-392.
Stepien RL, Hinchcliff KW, Constable PD, Olson J (**1998**) Effect of endurance training on cardiac morphology in Alaskan sled dogs. J Appl. Physiol 85: 1368-1375.
Thomas WP, Gaber CE, Jacobs GJ, Kaplan PM, Lombard CW, Moise NS, Moses BL (**1993**) Recommendations for standards in transthoracic two-dimensional echocardiography in the dog and cat.Echocardiography Committee of the Specialty of Cardiology, American College of Veterinary Internal Medicine. J Vet Intern Med 7: 247-252.
Vollmar AC (**1999**) Echocardiographic Measurements in the Irish Wolfhound: Reference Values for the Breed. J Am Anim Hosp Assoc 35: 271-277.
Vörös K, Hetye C, Reiczigel J, Czirok GN (**2009**) M-mode and two-dimensional echocardiographic reference values for three Hungarian dog breeds: Hungarian Vizsla, Mudi and Hungarian Greyhound. Acta Vet Hung 57: 217-227.
Yuill CD, O'Grady MR (**1991**) Doppler-derived velocity of blood flow across the cardiac valves in the normal dog. Can J Vet Res 55: 185-192.

Abbreviations

2D - two dimensional
A wave - late diastolic transmitral flow
Ao - aortic root diameter
bpm - beats per minute
d - diastole
E wave - early diastolic transmitral flow
EPSS - end point to septal separation
FS - fractional shortening
HR - heart rate
IVRT - isovolumetric relaxation time
IVS - interventricular septum
LA - left atrial dimension
LVD - left ventricular dimension
LVOT - left ventricular outflow tract
LVW - left ventricular free wall
MV - mitral valve
PA - pulmonary artery
RVD - right ventricular dimension
s - systole