ABSTRACT

OBJECTIVES: To establish transthoracic echocardiographic reference intervals in adult English Springer Spaniel dogs.

METHODS: Forty-two healthy adult English Springer Spaniels were prospectively recruited from a general practice population in the UK. Animals were examined twice, at least 12 months apart, to exclude dogs with progressive cardiac disease. Reference intervals were calculated using Box-Cox transformations and specific variables were depicted within an expert consensus range. Effects of body mass, age and heart rate on cardiac structure and function were examined. Functional assessments were compared to previous reports. Reference intervals were compared against published ratiometric indices and allometric scaling models.

RESULTS: Thirty-nine dogs were used to create reference intervals. Significant effects of body weight, age and heart rate were detected although low coefficients of determination were found. Fractional shortening values are lower than has been reported in many breeds however Simpson-derived ejection fractions were similar to previously published breed-specific values.

CLINICAL SIGNIFICANCE: Breed-specific reference intervals are reported allowing for more appropriate interpretation of echocardiographic assessments in the English Springer Spaniel.

INTRODUCTION

Quantification of cardiac chamber size and function is a key component of diagnostic echocardiography (Lang *et al.* 2015) however the diagnostic utility of cardiac imaging is dependent upon the use of appropriate reference intervals (RI). Whilst species-wide, allometrically-scaled and aorta-derived ratiometric RI are commonly used in dogs (Brown *et al.* 2003, Cornell *et al.* 2004), canine breed-specific RI are likely to be the more appropriate given the known differences in body mass, conformation and anatomy between breeds (Dukes-McEwan *et al.* 2003).

Like humans (Naylor *et al*. 2008), the canine heart physiologically remodels in response to exercise (Lonsdale *et al*. 1998, Stepien *et al.* 1998). These adaptations manifest as increases in chamber size and wall thickness, creating uncertainty when differentiating health from disease. Many dog breeds have been selectively bred for intense exercise and this could result in an “athlete’s heart” phenotype. The use of generic species-wide RI in sporting breeds will likely result in errors in interpretation and potentially lead to misdiagnosis (Bavegems *et al.* 2007, Jacobson *et al*. 2013, Seckerdieck *et al*. 2015). The development of breed-specific RI, especially in athletic breeds, could help reduce this uncertainty and improve clinical diagnoses.

English Springer Spaniels (ESS) are an athletic breed without established echocardiographic RI. The primary aim of our study was to establish echocardiographic RI in healthy adult English Springer Spaniels. Secondary aims were to examine the effect of body mass, age and heart rate on continuous echocardiographic variables; to evaluate if using species-wide allometric or aorta-based prediction intervals is valid in ESS and finally to compare Simpson’s-derived ejection fraction (EF) estimates in ESS to previously published values in other breeds.

MATERIALS AND METHODS

Animals

We prospectively recruited healthy English Springer Spaniel (ESS) dogs >18 months old from private practices in the UK. Ethical approval was granted (University of Cambridge Ethics and Welfare Committee reference CR111) and all owners provided informed consent. The first author performed all observations, examinations and measurements. Dogs were considered eligible for evaluation if they had an unremarkable history and normal physical examination, electrocardiogram (ECG) and Doppler-derived blood pressure (<160 mmHg). To exclude progressive myocardial disease, one investigator echocardiographically imaged all dogs twice at an interval of at least twelve months. Comprehensive echocardiographic examinations were performed at each evaluation, but only data from the second examination were used to create RI. By examining the dogs at two time points, at least twelve months apart, we were able to examine whether left ventricular internal dimension regressed to the mean. This analysis was conducted in order to examine cardiac health and rule out the potential confounding influence of undiagnosed myocardial disease. If our ESS represent a healthy population then any initial individual measurement above the mean would be expected to be lower than the mean 12 months later and *vice versa* (Rishniw *et al.* 2005). Animals were recorded as active gun dogs if they regularly performed (more than once weekly) training or work with live game during the shooting season (1st October to 1st February).

Image acquisition

Echocardiographic examination was performed using a GE Vivid Q (General Electric systems), a transducer with nominal frequency range 2.7 – 8 MHz with harmonic imaging and simultaneous ECG recording. Two-dimensional (2D), M-mode (MM), colour and spectral Doppler assessments were completed. Dogs were positioned, unsedated and gently restrained, in right and left lateral recumbency on a padded table with a cut-out for scanning from beneath.

Images were acquired according to published guidelines (Thomas *et al.* 1993) and a series of 9 cardiac cycles from each view were stored for later analysis. After the entire cohort had been imaged, examinations were analysed in a blinded manner within a 5-day period to minimise observer variability due to temporal drift (Gottdiener *et al.* 2004). The mean of three measurements was recorded for each value. All 2D and MM measurements were made using tissue-to-cavity interface: for measurements of the LV, end-diastole was defined as the largest LV dimension~~ close to QRS onse~~, immediately prior to systolic incursion of the septum ~~t~~ and systole as the smallest LV dimension (Lang *et al.* 2015). Heart rate was taken as the average of three R-R intervals recorded from the left ventricular MM trace.

Cardiac structure

The following linear dimensions were recorded from the right parasternal long-axis views: left atrial width (LA_{LAX}) at end-ventricular systole (last frame before mitral valve opening) (Rishniw and Erb 2000a); left ventricular length in diastole and systole (LVLd, LVLs). From the right parasternal short-axis views: systolic pulmonary artery diameter at valve level (PA) (Serres *et al.* 2007); left atrium and aortic root dimensions and ratio on the first clear frame after aortic valve closure in early diastole (LA_{SAX}, Ao, LA:Ao)(Rishniw and Erb 2000a); 2D and MM measurements of the interventricular septum, left ventricular lumen and left ventricular free wall in diastole and systole at the chordae tendineae level (IVSd, LVDd, LVFWd, IVSs, LVDs, LVFWs).

Doppler assessments

Maximal aortic flow velocity (Ao_{VMAX}) was recorded using continuous wave (CW) Doppler from the subcostal view. Trans-Mitral Doppler E-wave and A-wave maximal velocities (MV E, MV A) and E-wave deceleration time (MV E_{DEC}) were recorded with PW Doppler using the left apical four-chamber view (Quiñones *et al.* 2002). Valves were interrogated for regurgitation using colour flow mapping, with colour gain set just below speckling artefact, using optimised views depending on the valve. Regurgitation was subjectively classified as trace, mild, moderate or severe (Rishniw and Erb 2000b, Zoghbi *et al.* 2003, Jacobson *et al.* 2013). If tricuspid regurgitation was identified then maximal tricuspid regurgitation velocity (TR_{VMAX}) was recorded using CW Doppler. Maximal pulmonary artery flow velocity was recorded using PW Doppler (PA_{VMAX}) with the sample volume placed just distal to the pulmonic valve from the left cranial view. Angle correction was not used for any spectral Doppler recording.

Systolic function

Left ventricular volumes in diastole and systole were calculated using the monoplane Simpson's method of discs from the right parasternal long-axis view, optimised to the left ventricle (EDV, ESV)(Dukes-McEwan *et al.* 2003, Wess *et al.* 2010). E-point to septal separation (EPSS) was recorded using MM on the right parasternal long-axis inflow-outflow view (Holler and Wess 2013). Ejection fraction (EF) and fractional shortening (FS) were then calculated~~d according to published guidelines (O'Grady *et al.* 1986)~~. From the sub-costal view left ventricular pre-ejection period (PEP) was recorded as the time between the onset of the Q-wave until aortic valve opening recorded on the spectral trace; ejection time (ET) was recorded as the time between opening and closure of the aortic valve on the spectral trace (Quiñones *et al.* 2002) and LV PEP:ET was then calculated.

Indices of cardiac size

Left ventricular volume was indexed to surface area (Wess *et al.* 2010); left ventricular length was indexed to width (Sphericity Index)(Dukes-McEwan *et al.* 2003) and aortic-dimension-derived Echocardiographic Ratio Indices (ERIs) were calculated (Brown *et al.* 2003).

Observer variability

Intra-observer (measurement) variability was calculated from ten randomly selected examinations, expressed as the percentage difference between the two measurements divided by the mean (Smith *et al.* 2012, Georgiev *et al.* 2013). Left ventricular dimensions on M-mode, 2D LV volumes, LA:Ao and Ao_{VMAX} were measured twice, one week apart. Median observer variability was <5% and maximum variability <10% for all variables. As all images were acquired and measured by one investigator, inter-operator/observer variability was not addressed.

Statistical analysis

Statistical analysis was performed using PAST 3 software (Hammer *et al.* 2001). Outliers were identified using the r criterion (Friedrichs *et al.* 2012). Continuous echocardiographic variables were assessed for normality visually using histograms and formally using a Shapiro-Wilk test. We used 3 different methods to derive reference intervals. First, median, upper and lower reference limits and 90% confidence intervals of the limits were calculated within Reference Value Advisor (Geffré *et al.* 2011) using a robust method with a Box-Cox transformation, after confirming symmetric distribution. Next, a "trimmed range" was produced by removing the single highest and lowest values from the range. Finally, an "expert consensus" reference interval was created for certain echocardiographic variables: each author (who are all experienced canine or human echocardiographers) independently plotted upper and lower reference limits on scatter plots; the first author averaged these and the final plots were subsequently approved by all authors.

After confirming assumptions were not being violated, simple linear regression was used to assess relationships between body mass, heart rate and age with all continuous echocardiographic variables. Calculated ERIs were compared to published mean values using a Student's T-test. Measured MM LVDd and LVDs were plotted against predicted values from previously published species-wide allometric scaling models (Cornell *et al.* 2004). Simpson's-derived EFs were compared with previously published values in five other breeds. Alpha was set at $P<.05$.

RESULTS

Animals

Forty-two dogs met eligibility criteria for echocardiographic evaluation. Three were excluded due to gross cardiac abnormalities (two had degenerative mitral valve disease, one of which was excluded on the basis outlier analysis and the third a suspected persistent left cranial vena cava), leaving a study population of 39 dogs. The median interval between echocardiographic examinations was 17 months (ranging from 12 to 22 months). The study population comprised 24 males (nine neutered) and 15 females (seven neutered) with a median age of 5 years 8 months (2.5 to 12.8 years); mean weight of 19kg (13.2 to 26.1kg) and median body condition score of 4/9 (3 to 7/9). Five dogs were closely related. Pedigree details were not available for all study participants and we did not check for distant relationships. Fourteen dogs (36%) were active gun dogs. The echo examinations for the second study period were performed between 5th February and 16th August, 2015. One working dog was examined in February, six in March, three in April, one in May and three in August.

Mean heart rate was 100 beats per minute (bpm)(57 to 152bpm) and mean systolic blood pressure was 122mmHg (82 to 159mmHg). Seven dogs had murmurs detected on the second examination: all were graded as ”soft”(Ljungvall *et al*. 2015), audible over the left heart base in one dog and left apex in six. One dog with a murmur had mild mitral regurgitation, the remaining dogs had trivial or no mitral regurgitation.

Regression to the mean

Our study population demonstrated regression to the mean for MM LVDd and LVDs, with a negatively sloping line crossing the 0% change point and equal distribution of data around this point (FIG 1A and 1B).

Reference intervals

Several linear echocardiographic variables were not normally distributed. Unless stated, median values are reported. Descriptive statistics, a trimmed range and statistically-derived RI for 2D, MM, and Doppler echocardiographic values and indices are shown in Tables 1-3. “Expert consensus” reference intervals were plotted for six echocardiographic variables (EDV, ESV, MM LVDd, MM LVDs, MM IVSd, MM LVFWd) against bodyweight, with individual data points visible (FIG 2).

Valvular assessments

Valve morphology was unremarkable in all dogs however trace and mild regurgitations were frequently noted; 15 (38.5%) had trace and nine (23%) had mild mitral regurgitation; 15 (38.5%) had trace and five (13%) had mild tricuspid regurgitation; 20 (51%) had trace and two (5%) had mild pulmonary valve regurgitation. No dogs had aortic regurgitation. Where detected, TR_{VMAX} was ≤2.7m/s in all dogs.

Effects of body mass, age and heart rate

Significant, but weak, associations were detected between multiple echocardiographic variables and body mass, age and heart rate (Table 4).

Comparisons to published prediction models and other breeds

Mean values for LVDd:Ao and LVDs:Ao were significantly higher (P<0.0001) than values previously reported in a study of 53 dogs from a range of different breeds (Table 5). Diastolic and systolic LV dimensions were above the upper allometric 95% prediction intervals in 28% and 46% of animals, respectively (FIG 3 A&B). Measured EFs in ESS were similar to recently published values for five other breeds (Table 6).

DISCUSSION

Our results provide, for the first time, echocardiographic reference intervals for healthy adult ESS dogs from a UK population. Additionally, we examined the effects of body mass, age and heart rate on echocardiographic indices and compared our results to published generic and breed-specific data.

Previous investigators have suggested that dilated cardiomyopathy (DCM) has a high prevalence in ESS (Dukes-McEwan 2010). If ESS had a high prevalence of DCM, a high incidence of CHF and early cardiac death would be anticipated in this breed. However, in medium and large scale studies of DCM prevalence, ESS only accounted for between one and two percent of cases (O'Grady and O'Sullivan 2004, Martin *et al.* 2009). Furthemore, data from the Swedish insurance database show that the risk of cardiac mortality in ESS (under the age of 10 years) is lower than the overall population mean (relative risk = 0.726; Jens Häggström, personal communication). It is possible that previous investigators have interpreted the larger, rounder LV dimensions of the ESS heart as pathological, rather than evidence of genetic or physiological adaptation, and have thereby misclassified healthy ESS as diseased.

Many of the echocardiographic measurements in our population of ESS would be considered evidence of systolic myocardial dysfunction. For example, the lower reference limits for FS and EF are lower than the proposed diagnostic cut-offs for DCM (<20-25% for FS, <40% for EF, Dukes-McEwan *et al.* 2003). However, the median values and proposed RI for EF in ESS are very similar to values published for dogs from five other breeds, some of which are athletic (Table 6). A species-wide Sphericity Index <1.65 has previously been proposed to assist in diagnosing DCM (Dukes-McEwan *et al.* 2003); in ESS, the median Sphericity Index was 1.51 and the proposed lower reference limit was 1.23. In Doberman Pinschers, EDVI values of >95mls/m^2 and ESVI values of >55mls/m^2 are consistent with DCM (Wess *et al.* 2010); in ESS, the proposed upper reference limits of EDVI and ESVI were 114mls/m^2 and 68mls/m^2, respectively. Interestingly, these values are similar to recently published upper limits for EDVI and ESVI in two other athletic breeds: Whippets (109mls/m^2 and 55 mls/m^2) and Salukis (127mls/m^2 and 65 mls/m^2)(Seckerdieck *et al.* 2015). Therefore, ESS (and other athletic breeds) might have a different cardiac morphology from non-athletic breeds, such as

Doberman Pinscher, Great Danes, Irish Wolfhounds etc., and species-wide reference intervals or threshold values for measures of systolic myocardial function might not apply to athletic dogs.

The ESS in the present study were all described as active and healthy and 36% were working gun dogs. In human athletes, endurance training is known to increase left ventricular chamber size (Pelliccia *et al.* 1999, Abergel et al. 2004, Legaz Arrese *et al*. 2005, Naylor *et al.* 2008) and there is evidence that the same occurs in dogs (Lonsdale *et al.* 1998, Stepien *et al.* 1998) and specifically in ESS (van Israël *et al.* 2005). Whilst differentiating physiologic adaptation from pathologic remodeling can be a challenge (La Gerche *et al.* 2009), it is unlikely that people or animals with subclinical DCM would be able to perform at a high athletic standard. However, we acknowledge that further long-term evaluation of these dogs is required to definitively exclude disease and determine the influence of genetic adaptation and physiologic training on this population.

Limitations

Despite our best attempts at subject selection and screening, it is impossible to guarantee that our population did not include a few cases with preclinical cardiomyopathy: in some animals, twelve months might be insufficient to exclude static or very slowly progressive myocardial dysfunction. However we consider it unlikely that any of the dogs in our study had myocardial dysfunction for four reasons: first, dogs were selected randomly from a general dog population with a reported prevalence of DCM of only 1-2%; second, the distribution of data was unimodal, arguing against a sample which included overtly diseased dogs; third, the population demonstrated regression to the mean in two consecutive assessments taken at least 12 months apart – dogs with disease would be expected to progress or remain abnormal. Finally, DCM exhibits age-related prevalences (more common in older dogs)(Dukes-McEwan *et al.* 2003, Wess *et al.* 2010), but we found no relationships between age and left ventricular size or systolic function.

Measurements were made according to recent guidelines from human echocardiography (Lang *et al.* 2015): as such there may be subtle differences between our measurements and those made by other researchers using older equipment or techniques.

Some of the calculated reference intervals in our study exceed the actual measured range because the statistical method used predicts the reference intervals based on the population distribution: with small sample sizes these predictions can become unstable. The method we used (robust with a Box-Cox transformation) is preferred when the sample size is <40 and distribution is non-Gaussian (Friedrichs *et al.* 2012). The sample size in this study was relatively small (39 dogs): a minimum sample size of 120 has been previously recommended by the American Society of Veterinary Clinical Pathology as this allows for non-parametric analyses (Friedrichs *et al.* 2012). However, our sample population is greater than many studies providing breed-based echocardiographic reference intervals: a meta-analysis of 39 studies providing echocardiographic reference intervals from 1152 dogs found a median sample size of 20 dogs (minimum to maximum 11-144, Hall *et al.* 2008). Development and interpretation of RIs can be challenging and we have used three different methods to create RIs in ESS. Whilst the RIs derived from the statistical model are mathematically appropriate, the small sample size means that the confidence intervals are wide and this could create ambiguity in clinical situations. The trimmed range and “expert consensus” RIs provide a less conservative data set (Lang *et al.* 2015). Nevertheless, reference intervals derived from studies with fewer than 120 subjects should be interpreted cautiously.

Although we have reported EDVI and ESVI, these indices of cardiac dimension are controversial in human medicine (Dewey *et al.* 2008) and are yet to be widely studied in veterinary medicine, with RIs lacking for most breeds. It is mathematically inelegant and physiologically inappropriate to scale a volume (a three-dimensional variable) to a surface area (a two-dimensional variable) and, as both are related to body size, they will be proportional. Because of the wide diversity of body size in dogs, scaling to body volume (or mass) would be more appropriate, but this is rarely performed and does not account for changes in body condition. Our sample size did not allow for a robust analysis of breed-specific allometric scaling.

We did not detect statistically significant relationships between body mass and left ventricular chamber dimensions: this is likely due to our study being underpowered to detect a difference in this population, rather than because no difference exists. When we created the “expert consensus” reference interval plots, all authors independently set limits that accounted for a positive correlation between body mass and the measured variables. It is probable that a larger sample size would allow for the detection of subtle but significant relationships between echocardiographic variables and body mass. We specifically did not investigate effects of gender or working status on echocardiographic variables as our study was not adequately powered to address these issues. Whilst we have proposed that “athlete’s heart” can be a feature of the~~in~~ ESS breed, our data does not ~~confirm~~prove this and further research quantifying the effects of training on ESS is needed. Finally we did not fully exclude systemic disease from our population as no other diagnostic tests were performed.

Conclusions

Our study provides for the first time provisional echocardiographic reference intervals for the English Springer Spaniel breed. Specific variables, in particular indices of systolic function, differ widely from generalised guidelines or predicted intervals published elsewhere. English Springer Spaniels often appear to have rounded hearts with reduced systolic function at rest. This appears to be normal for the breed, may reflect an athletic phenotype and should not be immediately interpreted as evidence of subclinical myocardial dysfunction.

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FIGURE LEGEND

FIG 1A & 1B. Scatter plots showing regression to the mean of measured dimensions with equal distribution of data points above and below the horizontal line at 0, and a right-to-left downward sloping trend line.

FIG 2. Expert consensus reference intervals (blue zone) superimposed upon scatter plots for monoplane Simpson's derived left ventricular volumes (top row); M-mode derived left ventricular internal dimensions (middle row); M-mode derived left ventricular diastolic wall thicknesses (bottom row).

EDV end-diastolic volume, ESV end systolic volume, LVDd left ventricular internal dimension in diastole, LVDs left ventricular internal dimension in systole, IVSd interventricular septum in diastole, LVFWd left ventricular free wall in diastole, mls millilitres, BW bodyweight, kg kilogrammes

FIG 3 A&B. Scatter plots showing measured M-mode LVDd and LVDs, with 95% prediction intervals (dashed lines) using an allometric scaling method (Cornell et al. 2004).

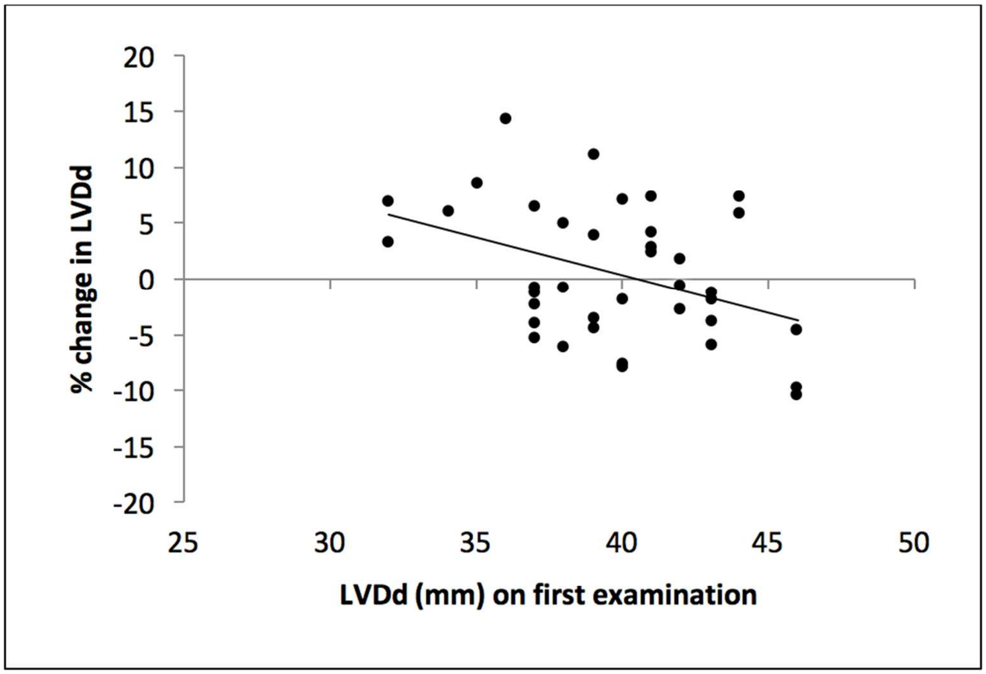

FIG 1A & 1B. Scatter plots showing regression to the mean of measured dimensions with equal distribution of data points above and below the horizontal line at 0, and a right-to-left downward sloping trend line.
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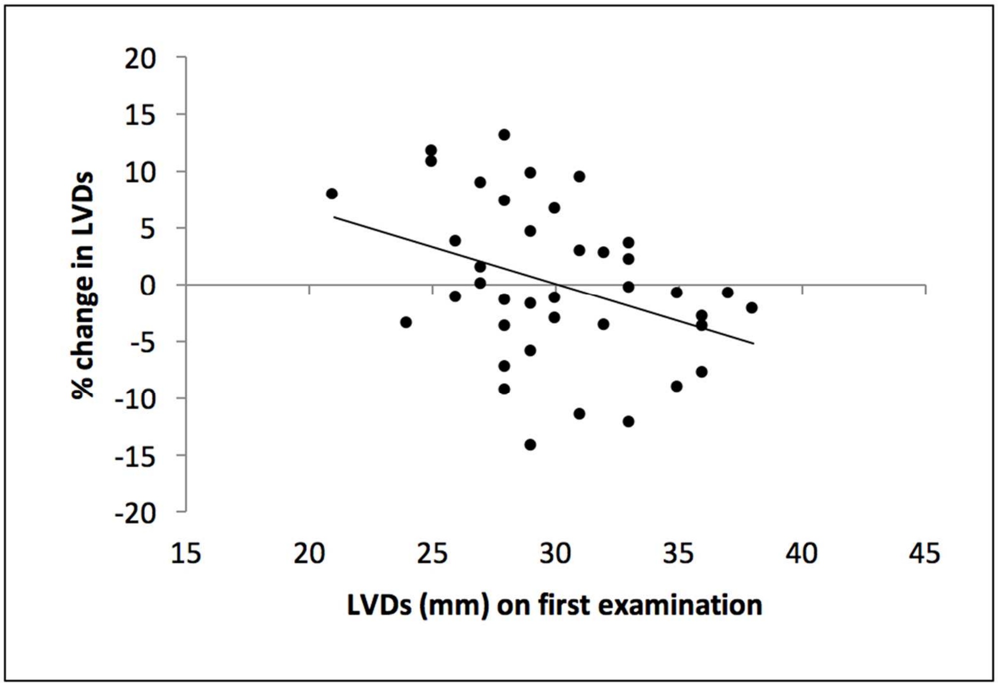

FIG 1A & 1B. Scatter plots showing regression to the mean of measured dimensions with equal distribution of data points above and below the horizontal line at 0, and a right-to-left downward sloping trend line.
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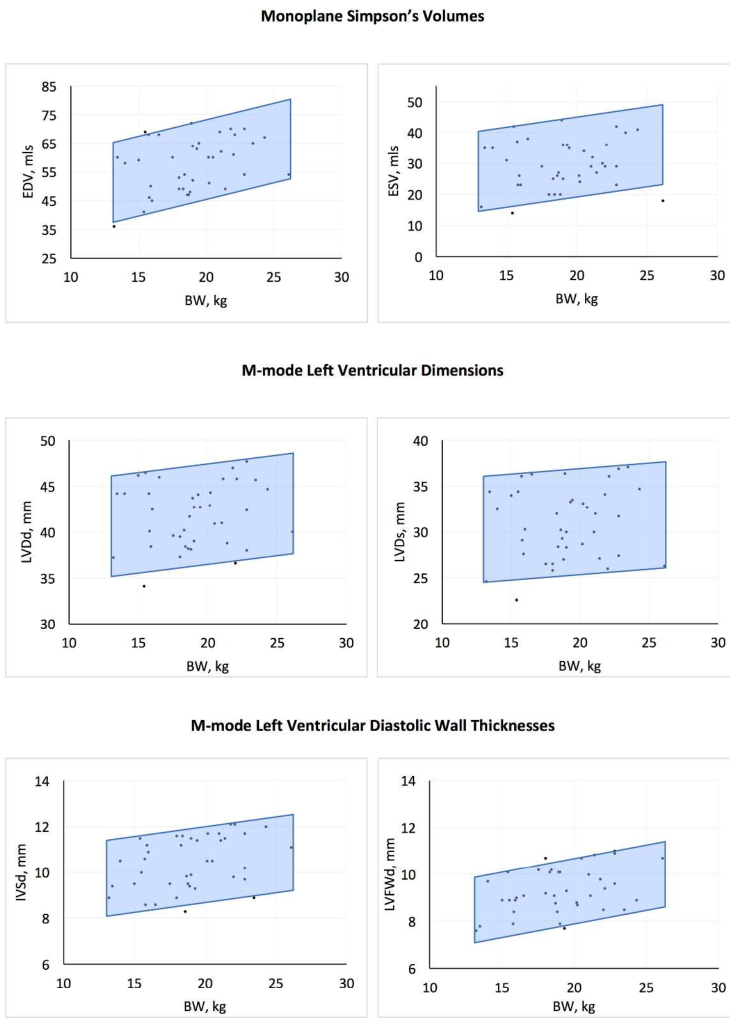

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EDV end-diastolic volume, ESV end systolic volume, LVDd left ventricular internal dimension in diastole, LVDs left ventricular internal dimension in systole, IVSd interventricular septum in diastole, LVFWd left ventricular free wall in diastole, mls millilitres, BW bodyweight, kg kilogrammes
209x296mm (300 x 300 DPI)

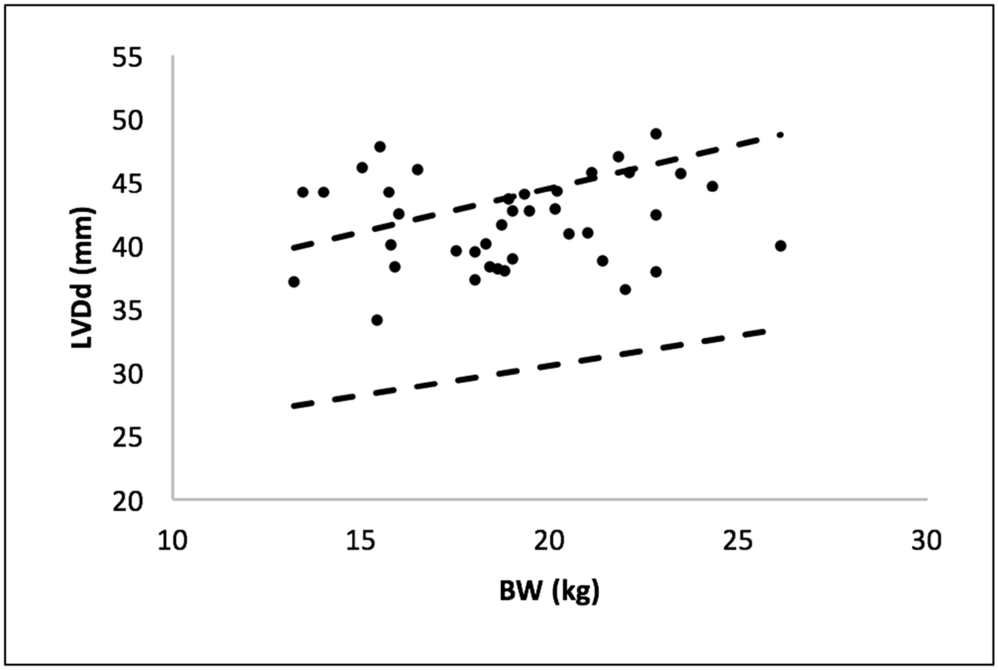

FIG 3 A&B. Scatter plots showing measured M-mode LVDd and LVDs, with 95% prediction intervals (dashed lines) using an allometric scaling method (Cornell et al. 2004).
356x240mm (72 x 72 DPI)

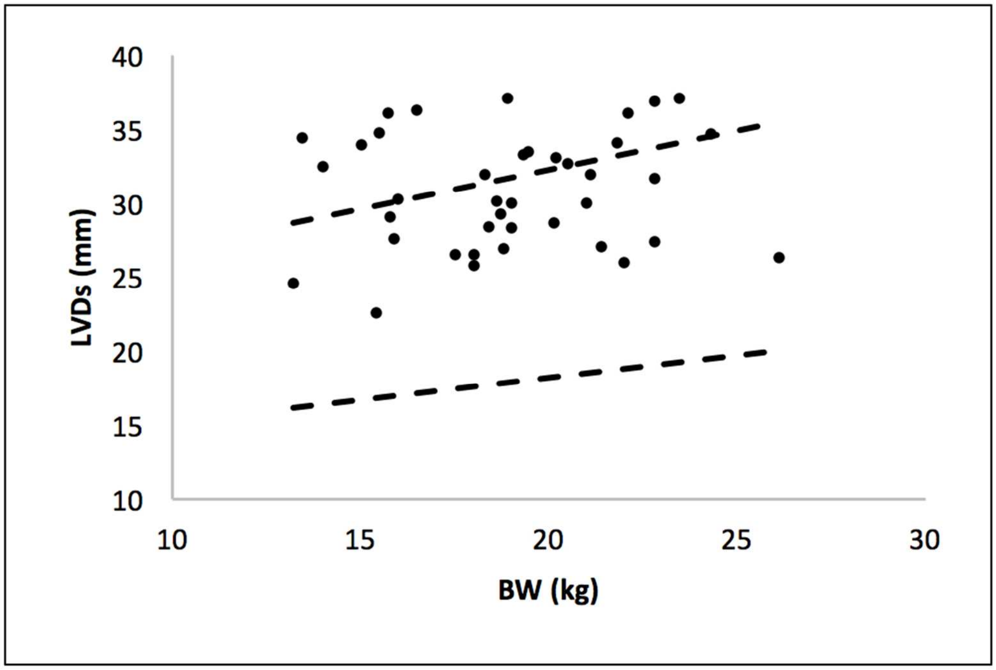

FIG 3 A&B. Scatter plots showing measured M-mode LVDd and LVDs, with 95% prediction intervals (dashed lines) using an allometric scaling method (Cornell et al. 2004).
357x239mm (72 x 72 DPI)

Table 1. Two-dimensional echocardiographic values in 39 healthy adult English Springer Spaniels

Mensural		Median	Min-Max	Trimmed range	Lower RI	(90% CI)	Upper RI	(90% CI)
LA_{LAX}	mm	36	30.5 – 42.7	32 – 42.2	30.7	(29.7 – 32)	42.5	(41 – 44.1)
LA_{SAX}	mm	29.2	23.6 – 36.1	23.7 – 35.3	23.3	(22.3 – 24.7)	36	(34.4 – 37.5)
Ao	mm	21.7	19.1 – 25.2	19.3 – 24.6	19.2	(18.8 – 19.8)	25.1	(24.2 – 26.1)
LA:Ao		1.33	1.04 – 1.62	1.09 – 1.62	1.06	(1.01 – 1.13)	1.65	(1.57 – 1.72)
PA	mm	17.6	15.8 – 20.7	16 – 20.3	15.6	(15.4 – 16.1)	21	(20.1 – 22)
PA:Ao		0.82	0.73 – 0.97	0.73 – 0.96	0.72	(0.7 – 0.74)	0.98	(0.93 – 1.03)
IVSd	mm	10.8	8.2 – 11.9	8.6 – 11.8	7.7	(5.8 – 8.7)	12.2	(12 – 12.5)
LVDd	mm	43.8	34.2 – 49.1	37.1 – 48.7	35	(33.7 – 36.6)	47.8	(46.7 – 48.5)
LVFWd	mm	9.6	7.4 – 10.8	7.6 – 10.7	6.5	(5.3 – 7.6)	10.9	(10.7 – 11.2)
IVSs	mm	13.1	9.3 – 16.3	10 – 15.9	9	(8.1 – 10.3)	16.2	(15.6 – 16.8)
LVDs	mm	32	24.2 – 37.5	24.7 – 37.2	22.6	(19.8 – 25)	39	(37.6 – 40.1)
LVFWs	mm	12.1	9.3 – 14.7	9.8 – 14.5	9.6	(9.1 – 10.2)	15	(14.3 – 15.7)
FS	%	25	17 – 38	17 – 36	16	(14.3 – 18.3)	35.9	(33.6 – 38.7)
LVDd:Ao		1.95	1.58 – 2.35	1.62-2.34	1.57	(1.49 – 1.67)	2.33	(2.25-2.42)
LVDs:Ao		1.44	1.09 – 1.94	1.15 – 1.8	1.1	(1.04 – 1.19)	1.86	(1.76 – 1.96)
LVLd	mm	64.3	51 – 69.9	55.9 – 69.4	53.2	(49.4 – 56.6)	70.8	(69.5 – 71.8)
LVLs	mm	54.3	47.9 – 60.7	48.1 – 60.4	47.5	(46.1 – 49)	61.5	(59.7 – 63.1)
Sphericity Index		1.49	1.17 – 1.81	1.18 – 1.77	1.19	(1.13 – 1.26)	1.83	(1.75 – 1.9)
EDV	mls	59	36 – 72	41 – 70	38.1	(34.2 – 43.3)	76.4	(72.5 – 79.6)
EDVI	mls/m^2	77.7	60.1 – 109	62.3 - 106	58.1	(55.2 – 61)	114	(104 – 125)
ESV	mls	29	14 – 44	16 - 42	14.2	(11.6 – 17.5)	46.2	(42.1 – 49.9)
ESVI	mls/m^2	37.6	20 – 66.3	22.2 – 60.8	20.6	(18.2 – 24.2)	68.1	(60.1 – 74.7)
EF	%	48	38.5 – 66.7	38.5 – 65.9	36	(34.3 – 38.1)	68.2	(62.9 – 73.8)
SV	mls	28	20 - 40	20 – 40	19.6	(18.4 – 21.4)	39.6	(36.4 – 43)

LA_{LAX} Left atrial internal long axis dimension, LA_{SAX} Left atrial short axis dimension, Ao Aortic short axis dimension, LA:Ao Short axis left atrial to aorta ratio, PA Pulmonary artery dimension, PA:Ao Pulmonary artery to aorta ratio, IVSd Interventricular septal thickness at end diastole, LVDd Left ventricular lumen dimension at end diastole, LVFWd Left ventricular free wall thickness at end diastole, IVSs Interventricular septal thickness at peak systole, LVDs Left ventricular lumen dimension at peak systole, LVFWs Left ventricular free wall thickness at peak systole, FS Fractional shortening, LVDd:Ao Left ventricular lumen dimension at end diastole to aorta ratio, LVDs:Ao Left ventricular lumen dimension at peak systole to aorta ratio, LVLd Left ventricular internal length at end diastole, LVLs Left ventricular internal length at peak systole, Sphericity Index (LVLd divided by LVDd), EDV End diastolic left ventricular volume, EDVI End diastolic left ventricular volume indexed to body surface area, ESV End systolic left ventricular volume, ESVI End systolic left ventricular volume indexed to body surface area, EF Left ventricular ejection fraction; SV Stroke volume

Trimmed range is the range minus the single highest and lowest values, RI Reference interval, CI Confidence interval, Min-Max Minimum-Maximum Reference intervals calculated using a robust method with a Box-Cox transformation (Geffré *et al.* 2011).

Table 2. M-mode echocardiographic values in 39 healthy adult English Springer Spaniels

Mensural		Median	Min-Max	Trimmed range	Lower RI	(90% CI)	Upper RI	(90% CI)
IVSd	mm	10.5	8.3 – 12.1	8.6 – 12.1	7.7	(6.9 – 8.3)	12.5	(12.2 – 12.9)
LVDd	mm	42.4	34.1 – 47.7	36.6 - 47	34.2	(32.4 – 36.2)	48.5	(47.1 – 49.7)
LVFWd	mm	9.1	7.6 – 11	7.7 – 10.9	7.4	(7.1 – 7.8)	11.4	(10.9 – 11.8)
IVSs	mm	13.3	10.1 – 17.7	10.6 – 17.5	9.9	(9.5 – 10.6)	18.1	(16.9 – 19.4)
LVDs	mm	30.3	22.6 – 37.1	24.6 – 36.9	22.9	(21.1 – 24.7)	38.8	(37 – 40.6)
LVFWs	mm	12.4	9.3 – 15.6	10.5 – 15.6	9.6	(9.1 – 10.2)	15.9	(15.1 – 16.7)
FS	%	27	17 – 35	19 - 34	17.3	(15.1 – 19.4)	35.8	(33.7 – 38)
EPSS	mm	7	2.7 – 9.5	3.1 – 9.2	1.5	(0 – 3.1)	10.5	(9.9 – 11.4)
LVDd:Ao		1.93	1.56 – 2.31	1.58 – 2.25	1.53	(1.44 – 1.63)	2.29	(2.21 – 2.37)
LVDs:Ao		1.41	1.1 – 1.8	1.11 – 1.72	1.02	(0.95 – 1.12)	1.81	(1.72 – 1.89)
Sphericity Index		1.51	1.23 – 1.88	1.26 – 1.82	1.23	(1.17 – 1.29)	1.88	(1.78 – 1.96)

EPSS E-point to septal separation. See table 1 for remainder of key.
Reference intervals calculated using a robust method with a Box-Cox transformation (Geffré *et al*. 2011).

Table 3. Doppler echocardiographic values in 39 healthy adult English Springer Spaniels

Mensural		Median	Min-Max	Trimmed range	Lower RI	(90% CI)	Upper RI	(90% CI)
Ao_{VMAX}	m/s	1.45	1.1 – 1.8	1.14 – 1.79	1.05	(0.98 – 1.14)	1.87	(1.78 – 1.95)
LV PEP	ms	72	47 – 95	51 - 93	48.3	(42.9 – 53.7)	95	(89.7 – 100)
LV ET	ms	160	124 – 198	139 - 186	133	(125 – 140)	187	(179 – 194)
LV PEP:ET		0.43	0.32 – 0.65	0.33 – 0.63	0.31	(0.29 – 0.33)	0.66	(0.6 – 0.73)
PA_{VMAX}	m/s	0.84	0.55 – 1.4	0.58 – 1.17	0.57	(0.52 – 0.62)	1.29	(1.14 – 1.44)
MV E	m/s	0.69	0.39 – 0.98	0.48 – 0.96	0.44	(0.38 – 0.5)	0.97	(0.9 – 1.04)
MV A	m/s	0.64	0.38 – 0.91	0.42 – 0.89	0.36	(0.32 – 0.42)	0.96	(0.89 – 1.03)
MV E:A		1.14	0.51 – 1.65	0.64 – 1.62	0.45	(0.3 – 0.63)	1.78	(1.66 – 1.89)

Ao_{VMAX} Maximal aortic flow velocity on continuous-wave Doppler; LV PEP Left ventricular pre-ejection period; LV ET Left ventricular ejection time; LV PEP:ET Left ventricular pre-ejection period indexed to left ventricular ejection time; PA_{VMAX} Maximal pulmonic flow velocity on pulsed-wave Doppler; MV E Mitral valve E wave velocity; MV A Mitral valve A wave velocity; MV E:A Mitral valve E wave to A wave ratio; m/s metres per second; ms milliseconds. See table 1 for remainder of key.

Reference intervals calculated using a robust method with a Box-Cox transformation (Geffré *et al.* 2011)

Table 4. Simple linear regression analysis of linear echocardiographic variables in healthy English Springer Spaniels

Dependent variable	Independent variable	Slope	Intercept	R^2	P value
LA_{LAX}	BW	0.389	28.8	0.179	0.007
LVLd	BW	0.760	49.3	0.319	0.000
EDV	BW	0.968	39.0	0.108	0.041
LVLs	BW	0.541	43.8	0.190	0.006
Ao	BW	0.183	18.4	0.157	0.012
IVSd	BW	0.140	7.91	0.180	0.007
$LVFWd_{2D}$	BW	0.136	6.72	0.202	0.004
$IVSs_{2D}$	BW	0.244	8.42	0.195	0.005
$LVFWs_{2D}$	BW	0.234	7.76	0.302	0.000
$IVSd_{MM}$	BW	0.130	7.96	0.127	0.026
$LVFWd_{MM}$	BW	0.122	6.99	0.158	0.012
$LVFWs_{MM}$	BW	0.241	7.99	0.244	0.001
MV E	Age (m)	-0.002	0.823	0.171	0.009
MV A	Age (m)	0.002	0.487	0.188	0.006
MV E:A	Age (m)	-0.006	1.584	0.328	<0.001
LVET	HR	-0.355	195	0.354	<0.001
MV E	HR	0.002	0.497	0.111	0.039
MV A	HR	0.002	0.399	0.134	0.022

BW Body weight in kilogrammes; Age (m) Age in months; HR heart rate; 2D Two-dimensionally-derived; MM M-mode-derived.
See table 1 for remainder of key.

Table 5. Comparison of measured ERIs in ESS and published values for mixed breed dogs

Mensural	ESS ERI Mean (SD)	Mixed breed ERI Mean (SD)	P value
LVDd:Ao (MM)	1.92 (0.19)	1.61 (0.20)	<0.0001
LVDs:Ao (MM)	1.41 (0.19)	1.06 (0.17)	<0.0001

ERI Echocardiographic Ratio Index; ESS English Springer Spaniel; LVDd:Ao Left ventricular lumen dimension at end diastole to aorta ratio, LVDs:Ao Left ventricular lumen dimension at peak systole to aorta ratio; MM M-mode; SD standard deviation.
Normally distribution was confirmed by Shapiro-Wilk test.
Data for mixed breeds from Brown *et al.* (2003).
Comparison of means with Student's T-test.

Table 6. Comparison of left ventricular ejection fractions in five different breeds of dog.

Breed	Reference	Ejection Fraction (%) Mean/Median	Ejection Fraction (%) Min-Max / Reference interval
English Springer Spaniel		48	36 – 68
Doberman	Wess *et al.* 2010	49	27 – 63
Boxer	Smets *et al.* 2013	49	35 – 63
Great Dane	Stephenson *et al.* 2012	54.5	42 – 63
Whippet	Seckerdieck *et al.* 2015	57	45 – 70
Saluki	Seckerdieck *et al.* 2015	52	41 – 64