Transthoracic echocardiographic mensuration of two-dimensional left atrial to aorta ratios and left ventricular M-mode parameters in clinically normal adult Dachshunds

by

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to Suk Lan

You are not only my wife but my best friend as well. You have stood with me in times of happiness as well as difficulties. Thank you so much for being part of my life.
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SUMMARY

Transthoracic echocardiographic measurements of two-dimensional left atrial to aorta ratios and left ventricular M-mode parameters in clinically normal adult Dachshunds

Lim CK, University of Pretoria, 2014

Dachshunds are predisposed to myxomatous mitral valve disease (MMVD) with a polygenic mode of inheritance for mitral valve prolapse. Changes in left heart geometry have been previously observed in dogs with chronic volume overload secondary to mitral regurgitation) in MMVD, particularly left atrial enlargement. The primary objectives of this study were to estimate normal values for the left atrium to aorta (LA/Ao) ratio and establish normal prediction intervals for left ventricular motion-mode (M-mode) transthoracic echocardiographic measurements in clinically normal adult Dachshunds.

The mean (standard deviation) for LA/Ao ratio measured in 40 clinically normal adult Dachshunds using the diameter, circumference and cross-sectional area standard two-dimensional methods via right parasternal short axis view were 1.40 (0.13), 2.19 (0.17) and 2.95 (0.48). The normal prediction intervals of the left ventricular measurements established via logarithmic transformation and linear regression in this study were found to have more narrow intervals than previous multi canine breed prediction intervals and were therefore more representative for clinically normal adult Dachshunds. The scaling exponents (b’) derived from this study ranged from 0.129 to 0.397 and did not absolutely conform to the presumed index of body length in the allometric equation, which is body weight raised to 1/3 power.

The 2-D LA/Ao ratios and M-mode left ventricular prediction intervals established from this study may be used as reference values for Dachshunds.
1.1 Background

In modern clinical advances, the invention of echocardiography is perhaps the most significant diagnostic modality for both human and veterinary cardiology. Echocardiography has been used clinically in veterinary medicine since the 1970s to provide detailed evaluation of the cardiac anatomy, function and haemodynamics that would otherwise require invasive techniques.\textsuperscript{1-3} Echocardiography can be performed by means of a transthoracic or transoesophageal approach. In veterinary medicine, transthoracic echocardiography is preferred mostly due to its feasibility of usage (does not require general anaesthesia) and relatively cheaper cost compared to transoesophageal echocardiography. Transoesophageal echocardiography has been developed in humans to overcome the limitations of transthoracic echocardiography, particularly in obese patients, during cardiac surgery and in cases where lung interference with the transthoracic sound beam is inevitable. The techniques and clinical applications of transoesophageal echocardiography in dogs have been reported.\textsuperscript{4} Conventional echocardiographic modalities include motion-mode (M-mode) and two-dimensional (2-D) echocardiography. The advent of Doppler echocardiography (colour flow and spectral) further enhances the versatility of echocardiography as it adds the ability to evaluate blood flow direction, pattern, velocity and peak pressure gradients within the heart and great vessels. Because of these abilities, echocardiography has supplanted cardiac catheterization as the preferred method for critically evaluating the heart in clinical veterinary medicine.\textsuperscript{5}

1.2 Problem statement

Echocardiographic parameters for dogs have been found to vary significantly between breeds. The allometric scaling (AS) method has been used to establish prediction intervals for left ventricular M-mode parameters in a multi canine breed study.\textsuperscript{6} In spite of correcting data for body weight (BW) or body surface area (BSA), several studies reported that breed and body conformation also influence canine
echocardiographic measurements. Additionally, changes in left ventricular geometry have been documented in dogs with chronic volume overload secondary to myxomatous mitral valve disease (MMVD). Dachshunds are predisposed to MMVD with polygenic mode of inheritance for the mitral valve prolapse (MVP) being suggested. The degree of left atrial enlargement is related to the severity of mitral regurgitation (MR), and therefore clinical assessment of the left atrial size is important in the evaluation of dogs with MMVD. To date, there are no published reference normal echocardiographic values for the Dachshund.

1.3 Research questions

This study was designed to answer the following questions:

“What are the values of the maximum upper limit of 2-D left atrium to aorta (LA/Ao) ratios for clinically normal adult Dachshunds obtained via transthoracic echocardiography?”

“Will the values of the maximum upper limit of 2-D LA/Ao ratios for clinically normal adult Dachshunds fit the reference values established by previous study?”

“What are the left ventricular M-mode prediction intervals for clinically normal adult Dachshunds obtained via transthoracic echocardiography?”

“Will the normal left ventricular M-mode prediction intervals established for clinically normal adult Dachshunds fit with the prediction intervals established by previous multi canine breed AS study?”

1.4 Hypotheses

i. 2-D LA/Ao ratios for clinically normal adult Dachshunds can be established using three standard measurement methods obtained via transthoracic echocardiography.

ii. The maximum upper limits of 2-D LA/Ao ratios established from this study will fit values established by a previous study.
iii. Left ventricular M-mode prediction intervals for clinically normal adult Dachshunds can be established by transforming BW (kg) and M-mode measurements (mm) using the natural logarithm and allowing for the implementation of the AS method using typical linear regression methods.

iv. Left ventricular M-mode prediction intervals established for Dachshunds differ from the prediction intervals established by a previous multi canine breed AS method.

1.5 Objectives

The main objectives of this study were to establish maximum upper limits of the 2-D LA/Ao ratios and to establish left ventricular M-mode prediction intervals for clinically normal adult Dachshunds via transthoracic echocardiography and to compare with previously established multi canine breed prediction intervals.

1.6 Benefits

Established LA/Ao ratios and left ventricular M-mode prediction intervals specifically for Dachshunds may be used as reference values when performing echocardiography in Dachshunds with suspect volume overload secondary to MMVD.
2.1 Introduction

2.1.1 Veterinary echocardiography

Echocardiography remains the standard and most commonly used non-invasive imaging tool for assessment of cardiac size and function in human as well as veterinary medicine.\(^{23,24}\) It is safe, widely available and relatively inexpensive. Amongst all cardiac imaging modalities, cardiac magnetic resonance imaging (MRI) has been widely acknowledged as the clinical gold standard for cardiac imaging in humans due to its excellent temporal and spatial resolution, the ability to acquire images in any desired plane, high degree of accuracy and reproducibility with regards to quantitative measurements.\(^{23,25-28}\) Although cardiac-gated MRI has been recently described as feasible in dogs\(^{29}\), the requirement of general anaesthesia and the inevitable alteration in normal cardiac values during anaesthesia has made cardiac-gated MRI less ideal for application in veterinary cardiology. Additionally, the technology is expensive and not all veterinary institutions have direct access to these modalities.

M-mode echocardiography refers to motion-mode echocardiography. This type of image displays the cardiac structures in a one-dimensional plane. The technical aspect of this echocardiographic mode has been extensively described.\(^{30}\) A simultaneous electrocardiographic tracing is often performed to serve as time reference of the cardiac cycle. In general, M-mode images are obtained in real-time, either from the short-axis plane of the left ventricle or from long-axis left ventricular outflow plane in a right parasternal location by placing the cursor over the structures of interest.\(^{31}\) Differences between the two approaches were small and within clinically acceptable limits in normal dogs in a previous study.\(^{32}\) Even though 2-D echocardiography has partly supplanted M-mode studies, M-mode echocardiography is still relatively useful and the easier mode of the two to obtain cardiac dimensions. Compared to 2-D echocardiography, M-mode echocardiography has a higher sampling rate which results in excellent temporal resolution, higher axial resolution and is superior to real-time images in detecting subtle changes in wall and valve motion.\(^{31,33}\) Accurate left ventricular M-mode measurements are obtained by aligning
the sampling line perpendicular to the short or long axis of the left ventricle but yet, in many dogs, this may be difficult to achieve. The introduction of anatomic M-mode (AMM) in newer generation ultrasound machines has largely resolved this difficulty and the operator can virtually align the sampling line based on the direct spatial orientation of the heart as seen on the 2-D image. This inherently reduces the variability of left ventricular measurements and also improves correlation with measurements made directly from the 2-D image. \(^{34,35}\) In clinical practice, AMM increases reproducibility as well as improves accuracy of measurements. \(^{33}\) Among the parameters commonly measured in M-mode to assess left ventricular function are left ventricular fractional shortening (FS), ejection fraction (EF), left ventricular internal diameter (LVID), left ventricular free wall thickness (LVFW) and E point to septal separation (EPSS). Fractional shortening is the difference in the left ventricular diastolic and systolic diameter expressed as a percentage of end-diastolic diameter and it provides indication to left ventricular systolic function. \(^{24}\) Fractional shortening is the most widely used echocardiographic index of left ventricular systolic function in veterinary patients as it is easy to measure. \(^{24}\) Normal FS for unsedated dogs is 27-48\% and 30-50\% depending on the breed. \(^{36,37}\) The distance between the anterior mitral leaflet E point and the left ventricular septal surface in systole is known as EPSS. \(^{38}\) The maximal initial opening of the anterior mitral valve leaflet (E point) is inversely related to the volume and rate of left atrial emptying and the left ventricular stroke volume. \(^{38}\) The measurement of EPSS has been used as a practical and easily reproducible clinical index of left ventricular function. \(^{38}\) An increased EPSS (normal < 6 mm) can be indicative of systolic dysfunction or mitral valve stenosis. \(^{38}\) Ejection fraction is a measure of the percentage of the end-diastolic volume ejected with each heartbeat. \(^{24}\) Normal dogs and cats should have an EF > 50\%. \(^{24}\) Clinically significant dilated cardiomyopathy cases typically have an EF < 40\%, a FS < 20\%, an increased EPSS and relative decreased left ventricular free wall thickness in diastole (LVFWd) when compared to left ventricular internal diameter in diastole (LVIDd). \(^{39}\) Contrary to this, MMVD cases would be expected to have an elevated FS because of the presence of increased preload, decreased afterload (due to a mitral leak) and increased contractile elements. \(^{40,41}\)

Standardized imaging planes and display conventions for 2-D echocardiography in the dog and cat have been recommended by The
Echocardiographic Committee of the Specialty of Cardiology, American College of Veterinary Internal Medicine. Compared to M-mode echocardiography, 2-D echocardiography provides easier viewing of the cardiac spatial anatomy and function. Two-dimensional assessment can be both qualitative and quantitative, and may provide a more global view of systolic function. When interpreting a 2-D echocardiogram, particular importance should be placed on size of chambers relative to each other. Two-dimensional echocardiography is generally superior in detection of cardiac masses, atrioventricular septal defects, regional wall disorders, some congenital lesions and heartworms. It is also useful in detecting valvular diseases (eg: MMVD, vegetative valvular endocarditis), pericardial effusion, cardiomyopathies (eg: dilated cardiomyopathy and hypertrophic cardiomyopathy), heart base tumours and intracardiac tumours (eg: haemangiosarcoma).

2.1.2 Left atrium to aorta ratio, breed specific echocardiographic reference values and allometric scaling prediction intervals for all canine breeds

There is adequate evidence that suggests that an enlarged left atrium is indicative of significant ventricular, atrial or valvular disease and is a marker of adverse cardiovascular outcomes in humans. Two-dimensional echocardiography is the most widely accepted non-invasive imaging modality to assess left atrial size. Methods for assessing left atrial size in dogs using 2-D echocardiography and M-mode echocardiography have been evaluated extensively in veterinary studies. The diameter, circumference and cross-sectional area methods using 2-D echocardiography via the right parasternal short axis (RPSA) view have been proposed to be better standardized measurements of left atrial size. The same study also established the normal 2-D echocardiographic reference intervals for clinically normal dogs with a LA/Ao ratio of < 1.6 using the diameter method, < 2.46 using the circumference method and < 3.86 using the cross-sectional area method. It was concluded that the measurement of the left atrium using 2-D echocardiography via RPSA view at the level of the aortic cusps and normalized to the aorta size (LA/Ao ratio) was more accurate and sensitive than the M-mode echocardiographic measurement taken from the RPLA view in detecting left atrial enlargement.
To date there are approximately 20 publications on breed-specific normal echocardiographic reference values. These studies have established reference values for 4 giant breeds, 11 large breeds, 1 medium breed, 3 small breeds and 1 toy breed. There are also many studies that indirectly provide some normal echocardiographic reference values for certain dogs. However, some critics have questioned the usefulness of some of these reference ranges due to small sample sizes. As a result, various methods of statistical analysis such as AS and ratio indices have been proposed to predict reference values that may be applicable to all canine breeds using data from larger number of dogs from multiple breeds and variable sizes. In 2004, the AS method was adapted for predicting normal M-mode reference ranges for all dogs. The AS prediction intervals were calculated based on the M-mode values from 494 dogs from eight known breeds and some mixed breeds. The AS equation takes the form of $Y = aM^b$ where $Y$ represents each particular M-mode value, $M$ is BW, $a$ is the proportionality constant and $b$ the scaling exponent. The established prediction intervals were claimed to be applicable to most adult dogs ranging from BW of 2.5-90 kg regardless of breed. In spite of the corrected data for BW or BSA, several other studies also reported that body conformation influences canine echocardiographic measurements. These studies recommended that breed and conformation be considered when establishing normal echocardiographic reference ranges.

2.2 Myxomatous mitral valve disease and Dachshunds

Myxomatous mitral valve disease is the most common heart disease in small-breed dogs and is characterized by myxomatous valvular degeneration. Myxomatous mitral valve disease has many synonyms, including degenerative mitral valve disease, endocardiosis and chronic valvular disease. Myxomatous mitral valve disease has been extensively described in Cavalier King Charles Spaniels as well as in Dachshunds. A large scale retrospective study involving 942 dogs of six breeds of small-sized dogs (excluding Cavalier King Charles Spaniel) have found that Dachshunds and Shih Tzus were the most predisposed to mitral valve disease. Although the exact cause of canine MMVD has not been ascertained, its particular affiliation with certain breeds suggest that
genetic factors play a major role.95 Another large scale study involving 190 Dachshunds which also included 31 parents and 92 offspring has indeed found that MVP in Dachshunds is an inherited condition, most likely due to polygenic mode of inheritance.17 The same study also found that there is a negative correlation between the thoracic circumference of the Dachshunds and MVP. In humans, asthenic habitus (eg: low anteroposterior thorax diameter) is also associated with marked increased risk of MVP.96-98 Hence, it was hypothesised that the relatively small thoracic circumference and slightly rounded thoracic conformation in Dachshunds may have led to entrapment of the heart within the thorax and predisposing them to MVP and MMVD. The prevalence of MVP among young and old, clinically healthy Dachshunds without heart murmur was relatively high at 47% while a later study recorded a much higher prevalence of 81%.17,18 The higher percentage of MVP in the latter study is likely due to lesser interobserver variability because the diagnosis of MVP was made by a single observer. Several studies have documented MVP as an important component of developing MMVD.19,92,99-101 Following a vicious cycle, the abnormal valve motion due to the prolapsed valvular leaflets increases the shear stress imposed on the valves directly through abnormal leaflet coaptation and indirectly through increased regurgitant flow.18,92 With time, the valvular endothelial damage or loss progresses further and concomitantly the severity of valvular prolapse, myxomatous degeneration of the valves and regurgitant flow also increases.90 Mitral regurgitation caused by MMVD has been reported to account for up to 75% of canine cardiac diseases.90 The severity of the MR can be semiquantitatively measured using colour Doppler echocardiography by measuring the jet size against the size of the left atrium.102-104 A maximum regurgitation jet area to left atrial area (RJA/LAA) < 20%, between 20-40% and > 40% is classified as mild, moderate and severe MR respectively.102 It is important to emphasize that small regurgitant jets in the vicinity of the mitral valve should not be overinterpreted in dogs without any other valvular abnormality as these are considered as physiological regurgitation that can be often detected in normal dogs.90,105 Mitral regurgitation secondary to MMVD has been found to be the most common cause of left atrial enlargement in small breed dogs, particularly Cavalier King Charles Spaniels.89,99,106 A large scale prospective study involving 558 dogs of variable sizes with combination of MVP and MMVD from 36 breeds reported the LA/Ao ratio of > 1.7 and mitral valve E peak flow > 1.2 m/s as significant variables for all causes of cardiac related death
in these animals. A left atrium to aorta ratio of > 1.7 using the diameter method was specifically noted as a significant variable to predict cardiac-related deaths. The normal LA/Ao ratio for Cavalier King Charles Spaniels was 1.03 ± 0.09 using 2-D echocardiography diameter method via RPSA view at the level of the aortic cusps, markedly less than the upper limit of 1.6 described by a previous study. In spite of numerous large scale studies on mitral valve prolapse in Dachshunds, there are no published reference LA/Ao ratios for this breed to date.

The faulty valves in MMVD leads to MR and result in increased total stroke volume as the blood is ejected in a forward direction into the aorta and retrograde into the left atrium. The resultant vicious cycle of recirculation of blood from the left ventricle retrograde into the left atrium and back into the left ventricle again leads to low-pressure volume overload. In response to the stretching of the left ventricle by low grade volume overload, the extracellular matrix of the myocardium undergoes remodelling. The cardiomyocytes that are exposed to abnormal stress and strain pattern during the cardiac cycle from increased preload will undergo remodelling with loss of collagen weave, stretching and hypertrophy, resulting in so-called eccentric hypertrophy. As the mitral valve disease progresses, the severity of MR increases and further changes in left ventricular geometry ensue with chronic volume overload. Another study has also found that small breed dogs with chronic mitral valvular insufficiency had a significantly increased FS and end-systolic volume index but only two Dachshunds were represented in the study. In a separate study, LVIDd and N-terminal pro B-type natriuretic peptide concentration were found to be significant in predicting mortality in dogs with MMVD. The greater the values of each variable from normal range, the higher the risk of death. The predisposition of Dachshunds to MVP and MMVD emphasizes the need for normal echocardiographic reference values for this breed to be established, particularly for future comparison and monitoring purposes.
Chapter 3: MATERIALS AND METHODS

3.1 Study design

3.1.1 Sample size

This study was a prospective, minimally invasive experiment involving a total of 55 adult Dachshunds (26 males and 29 females).

3.1.2 Animal selection

3.1.2.1 Animal sourcing

Fifty-five Dachshunds from 1 to 7 years of age, not of toy size according to Federation Cynologique Internationale, owned by local breeders, clients or faculty staff that were deemed clinically healthy were recruited for this study. A pet owner-friendly information brochure was sent out to local Dachshund breeders as well as to clients and faculty staff that owned Dachshunds to create awareness regarding polygenic inheritance of MVP in Dachshunds as well as predisposition, diagnosis and medical management of MMVD (Appendix A). All participating Dachshunds received at least one complementary physical examination and complete echocardiographic examination.

3.1.2.2 Preliminary screening

A complete physical examination paying particular attention to the cardiovascular and respiratory systems (documenting habitus, temperature, pulse, respiration, hydration, mucous membrane colour and detailed cardiac auscultation) was performed. The gender, neutering status, body condition score (BCS) (using 5-point scale) and girth were also recorded (Appendix B). In order to increase likelihood of diagnosing a dog with a mild murmur, cardiac auscultation was performed after a mild stress test (e.g., after dogs running 20 meters). Cursory echocardiography was performed specifically to interrogate the mitral valve for identification of MVP using the right parasternal long-axis (RPLA) 4-chamber view as described in previous studies. An Aloka Prosound F75 ultrasound machine (Hitachi Aloka Medical, Tokyo, Japan) using a phased array transducer (frequency...
2.6-7.7 MHz) was used and a 5-second cine-loop was recorded to assess the motion of the mitral valve leaflets in real time and frame by frame. The hinge points of the two mitral valve leaflets were used to define the plane of the mitral annulus. The maximal degree of protrusion of any point of the mitral leaflets (including the anterior leaflet, posterior leaflet and coaptation point) into the left atrium during systole (with aid of cine-loop) was measured relative to the annulus plane. Leaflets that did not reach the annulus plane were given a negative value. The MVP severity (using the average of three measurements) was defined as ≤ 0 mm = no MVP; > 0 mm but ≤ 1 mm = mild MVP; > 1 mm = moderate to severe MVP (Appendix C).\textsuperscript{20}

Colour Doppler echocardiography was used to detect mitral regurgitation on the left apical 4-chamber view (Appendix B). Animals with MVP > 1 mm or MR were excluded from the study. A complete echocardiogram was performed for excluded animals to aid in potential medical management for these patients. Advanced screening (data capture) of selected animals for this study was performed within six hours after preliminary screening.

3.1.2.3 Advanced screening

Animals that passed the preliminary screening were subjected to thoracic radiography, complete blood count (CBC), peripheral blood smear and electrocardiographic (ECG) examination prior to complete echocardiography for inclusion in this study. Right lateral recumbency (RLR) and dorsoventral (DV) thoracic radiographs were made of each dog within 48 hours prior to complete echocardiographic examination at Onderstepoort Veterinary Academic Hospital. Complete blood count (measured using Cell' Dyn® 3700, California, USA) and peripheral blood smear was performed within 24 hours prior to the complete echocardiographic examination (Appendix B). A resting ECG (Nihon Kohden® Cardiofax GEM ECG-9020K, Tokyo, Japan) was recorded in RLR for five minutes (using bipolar and augmented unipolar limb leads) and three repeated measurements of indirect systolic blood pressure (SBP) using a standard Doppler technique were recorded within one hour prior to echocardiographic examination\textsuperscript{111} (Appendix D). Inclusion criteria were as follows: no abnormal findings on physical examination or history, no echocardiographic evidence of mitral valvular disease, no MVP or MVP ≤ 1 mm, no MR, no significant pulmonary disease detected.
radiographically, and no echocardiographic, radiographic or ECG evidence of congenital and/or acquired heart disease.

3.2 Transthoracic echocardiography

3.2.1 Echocardiography data acquisition

3.2.1.1 Animal preparation

Body weight, age, gender and neutering status were recorded prior to commencement of the scan. No sedation or general anaesthesia was used for the echocardiographic examinations. All animals were clipped on both sides of the thorax to minimize the effects of air trapped in hair on sound transmission. The ventral half of the right 3rd to 6th intercostal spaces and the ventral half of the left 4th to 7th intercostal spaces were clipped from the costochondral junction to the sternum using an Oster® clipper with size 40 blade. Five minutes were allocated for each animal to acclimatise to the room environment prior to the echocardiographic procedure. Ultrasound coupling gel was applied to the clipped area to enhance the skin-transducer contact. The heart rate was recorded during the scan using data derived from the ECG tracing.

3.2.1.2 Animal positioning

A Plexiglas scanning table with two cut-outs to allow imaging from below the table was used. The animal was placed in RLR with the right 3rd to 6th intercostal spaces positioned over a cut-out section in the table to allow scanning via the right thoracic wall from beneath the table. The animal was gently restrained by standing behind its back and placing the restrainers’ arms over the animal’s hips and neck while holding the legs. This was followed by placing the animal in left lateral recumbency with the 4th to 7th intercostal spaces positioned over another cut-out section in the table to allow scanning via the left thoracic wall from beneath the table. A similar gentle restraining technique was applied.
3.2.1.3 Echocardiographic scanning equipment

An Aloka Prosound F75 ultrasound machine (Hitachi Aloka Medical, Tokyo, Japan) using a phased array transducer (frequency 2.6-7.7 MHz) equipped with AMM software was used to perform the transthoracic echocardiography. Each animal was attached to three ECG electrodes and a simultaneous lead II ECG tracing was recorded and superimposed on the display monitor. End-diastole was defined as the onset of QRS complex while end-systole was defined as the maximum excursion of the interventricular septum (IVS). All echocardiographic examinations were performed by the primary investigator (CKL).

3.2.2 Echocardiography data recording

3.2.2.1 Two-dimensional (2-D) echocardiography

A complete 2-D echocardiographic examination was performed, in accordance to standard recommendations by The Echocardiographic Committee of the Specialty of Cardiology, American College of Veterinary Internal Medicine. Using the right parasternal window, the RPLA 4-chamber view, RPLA left ventricular outflow view and RPSA views at the level of the left ventricular apex, papillary muscle, chordae tendineae, mitral valve, aorta and pulmonary arteries were obtained. Using the left parasternal window, the left parasternal apical 4-chamber view and left parasternal apical 5-chamber (left ventricular outflow) view were obtained. The combination of these standard 2-D echocardiographic views in real-time provided realistic and understandable anatomic imaging of the animal’s heart and allowed screening for potential cardiac masses, atrioventricular septal defects, gross valvular (atrioventricular valves and semilunar valves) lesions, regional wall disorders and other congenital lesions.

The maximal thickness of the mitral anterior septal leaflet and posterior septal leaflet was measured in early systole (1\textsuperscript{st} frame after closure) in the RPLA 4-chamber view.

The diameter of the LA and the Ao were measured using the standard RPSA view at the level of the aortic valve where the commissures of the valve cusps were visible using a standard method described earlier (Fig. 1). The internal short-axis
diameter of the aorta along the commissure between the non-coronary and right coronary aortic valve cusps was measured on the 1st frame after the aortic valve closure while the left atrium chamber size was obtained by measuring the line extending from and parallel to the commissure between the non-coronary and left coronary aortic valve cusps to the distant margin of the left atrium in the same frame (Fig. 1). In images where a pulmonary vein entered the left atrium at the caudolateral location, the edge of the left atrium was approximated by joining the visible edges of the left atrium in an imaginary curved line.

**FIG. 1.** Yellow dotted line represents the internal short-axis diameter of the aorta along the commissure between the non-coronary and right coronary aortic valve cusps. This was measured on the 1st frame after aortic valve closure. * represents a pulmonary vein entering the left atrium at the caudolateral location. Green dotted line represents the line extending from and parallel to the commissure between the non-coronary and left coronary aortic valve cusps to the distant margin of the left atrium (the imaginary edge between the visible margins of the left atrium) in the same frame. The LA/Ao ratio was obtained by dividing the diameter of the left atrium by the diameter of the aorta.

The circumference of the LA and the Ao were obtained by manually tracing the internal short-axis circumferences of the left atrium and aorta from the same frame.
used to measure the diameter. These measurements were calculated automatically by the ultrasound machine software (Fig. 2).

**FIG. 2.** The internal short-axis circumference of the left atrium (continuous red line) and the aorta (continuous yellow line) were traced manually and measured automatically by the ultrasound machine software. The LA/Ao ratio was calculated by dividing the circumference of the left atrium by the circumference of the aorta. * represents a pulmonary vein entering the left atrium at the caudolateral location.

The cross-sectional area of the LA and the Ao were calculated by the ultrasound machine software after measuring the internal short-axis circumferences of the left atrium and aorta (Fig. 3).

**FIG. 3.** Using the same frame as above the cross-sectional area of the left atrium (red) and the cross-sectional area of the aorta (yellow) were calculated using the ultrasound machine software after measuring the internal short-axis circumferences of the left atrium and aorta. The LA/Ao ratio was obtained by dividing the cross-sectional area of the left atrium by the cross-sectional area of the aorta. * represents a pulmonary vein entering the left atrium at the caudolateral location.
Three individual measurements of each variable were acquired in different but not necessarily consecutive heart cycles. All measurements were automatically recorded and averaged by the built-in software within the ultrasound machine (Appendix E).

### 3.2.2.2 Motion-mode (M-mode) echocardiography

All M-mode measurements of the left ventricle were obtained simultaneously with B-mode (2-D) real time display in a short-axis view at the chordal level, immediately below the mitral valve. Anatomic M-mode was used in order to align the sampling line to accurately bisect the left ventricular chamber equally between the two papillary muscles (Fig. 4). Three individual measurements of interventricular septum thickness in diastole (IVSd), interventricular septum thickness in systole (IVSs), left ventricular free wall thickness in diastole (LVFWd), left ventricular free wall thickness in systole (LVPWs), left ventricular internal diameter in diastole (LVIDd), and left ventricular internal diameter in systole (LVIDs) were measured in millimetres (mm) while the FS and EF were calculated in percentages (%) (Appendix F). A simultaneous lead II ECG was recorded to ensure that the R-R interval for the three repeated measurements did not differ by more than 20%. All M-mode measurements were acquired using the leading-edge of myocardial borders\(^{112}\) (Fig. 5).

E point to septal separation was measured from the maximum opening of the septal mitral valve leaflet in early diastole to the interventricular septum.\(^{38}\)

Ejection fraction and FS were calculated using automated calculation software provided within the ultrasound machine.
FIG. 4. Left image: Anatomic M-mode was used to improve accuracy of left ventricular M-mode measurements by aligning the sampling line (solid line) perpendicular to the short axis of the left ventricle and consistently bisecting the left ventricle in two equal halves.

FIG. 5. Left image: Anatomic M-mode was used to align the sampling line (solid line) perpendicular to the short axis of the left ventricle in order to bisect the left ventricle in two equal halves. Right image: M-mode measurements of IVSd, interventricular septal thickness in diastole; IVSs, interventricular septal thickness in systole; LVIDd, left ventricular internal diameter in diastole; LVIDs, left ventricular internal diameter in systole; LVFWd, left ventricular free wall thickness in diastole; LVFWs, left ventricular free wall thickness in systole, were measured in millimetres (mm) using the leading edge method.111
3.4 Data and statistical analyses

All data were presented as mean ± standard deviation (SD). The 95% prediction intervals were defined as the range of values ± 2SD from the mean difference.

Data distributions for the LA/Ao ratios, the left ventricular M-mode measurements, EF, FS and EPSS were described by calculating the mean, SD, range (minimum, maximum), and an approximate 95% prediction interval (mean ± 2SD). Body weight (kg) and M-mode measurements (mm) were transformed using the natural logarithm to allow for the allometric scaling using typical linear regression methods. New proportionality constants (a'), scaling exponents (b'), and 95% prediction intervals were estimated by fitting a linear regression using each log-transformed M-mode measurement as the dependent variable and the log-transformed body weight as the independent variable. The importance of factors including age, gender, neutering status, BCS and girth on the prediction of normal M-mode measurements were assessed by adding each variable one-by-one to the AS regression model. The predictive ability of each variable was assessed through the regression coefficient, P value, and change in the coefficient of determination (r²) over the simple AS model. The validity of the previously published multi canine breed AS method⁶ was assessed by calculating the proportion of observed M-mode values from the current study that were contained within the 95% prediction intervals, estimating the Pearson’s correlation between observed values and predicted values based on the previous study, performing paired t-tests comparing observed and predicted values, creating scatter plots of observed and predicted values, and creating modified Bland-Altman plots depicting the bias of predicted values. The bias in the Bland-Altman plot was calculated using the formula: Measured value – predicted value of multi canine breed AS study⁶. All statistical analyses were performed in commercially available software (MINITAB Statistical Software, Release 13.32, Minitab Inc, State College, Pennsylvania, USA) and results interpreted at the 5% level of significance.
3.5 Ethical considerations

The trial was approved by the Animal Use and Care Committee of the University of Pretoria prior to commencement of this study (Protocol No.V061/11). Owners’ written consent was obtained for all procedures included in this study (Appendix G).
Chapter 4: RESULTS

4.1 Study population

A total of 55 adult Dachshunds were recruited. Mitral valve prolapse was observed in 25 Dachshunds (45.5 %), in which 15 of the 25 dogs were excluded from the study due to MVP > 1 mm or MR. Of the 15 dogs excluded from the study, one dog also had mild mitral regurgitation (30 % regurgitant jet of the left atrial area) and two dogs had minimal mitral regurgitation (RJA/LAA < 10 %) while one dog had a concomitant aortic aneurysm. Forty dogs fulfilled the inclusion criteria, comprising 16 males (five neutered) and 24 females (eight neutered). The mean (range) age was 3.7 (1, 7) years and the mean (range) weight was 8.5 (5, 12.6) kg (Table 1).

4.2 Descriptive statistics

The mean value, mean +/- 2SD and the range of the body temperature, heart rate, respiratory rate, SBP and BCS at the time of echocardiographic examination, as well as the LA/Ao ratios for the three measurement methods and M-mode measurements of the left ventricle and the FS, EF and EPSS are presented in Table 1.
<table>
<thead>
<tr>
<th>Measurement</th>
<th>Mean (SD)</th>
<th>Mean+/- 2SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>3.7 (1.6)</td>
<td>0.4, 7</td>
<td>1, 7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>8.5 (2.1)</td>
<td>4.3, 12.6</td>
<td>5, 12.6</td>
</tr>
<tr>
<td>Heart rate (/min)</td>
<td>113 (22)</td>
<td>69, 157</td>
<td>60, 160</td>
</tr>
<tr>
<td>Respiratory rate (/min)</td>
<td>37 (12)</td>
<td>13, 61</td>
<td>20, 64</td>
</tr>
<tr>
<td>Body condition score (5-point)</td>
<td>3.1 (0.6)</td>
<td>1.9, 4.3</td>
<td>2, 4.5</td>
</tr>
<tr>
<td>LA/Ao ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter method</td>
<td>1.40 (0.13)</td>
<td>1.14, 1.66</td>
<td>1.19, 1.65</td>
</tr>
<tr>
<td>Circumference method</td>
<td>2.19 (0.17)</td>
<td>1.85, 2.53</td>
<td>1.75, 2.42</td>
</tr>
<tr>
<td>Cross-sectional area method</td>
<td>2.95 (0.48)</td>
<td>1.99, 3.91</td>
<td>1.87, 3.86</td>
</tr>
<tr>
<td>M-mode (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVSd</td>
<td>6.4 (0.8)</td>
<td>4.8, 7.9</td>
<td>4.6, 7.8</td>
</tr>
<tr>
<td>IVSs</td>
<td>8.7 (1)</td>
<td>6.7, 10.7</td>
<td>6.7, 10.8</td>
</tr>
<tr>
<td>LVIDd</td>
<td>27.7 (3.4)</td>
<td>20.8, 34.5</td>
<td>21.6, 34.5</td>
</tr>
<tr>
<td>LVIDs</td>
<td>16.3 (3)</td>
<td>10.4, 22.2</td>
<td>11.1, 21.1</td>
</tr>
<tr>
<td>LVFWd</td>
<td>6.7 (0.9)</td>
<td>4.9, 8.4</td>
<td>5.2, 8.6</td>
</tr>
<tr>
<td>LVFWs</td>
<td>9.9 (1.2)</td>
<td>7.5, 12.3</td>
<td>7.2, 12</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>73.(5.9)</td>
<td>62, 85.6</td>
<td>61.3, 85.9</td>
</tr>
<tr>
<td>Fractional shortening (%)</td>
<td>41.4 (5.1)</td>
<td>31.2,51.6</td>
<td>31.6, 53.4</td>
</tr>
<tr>
<td>E point to septal separation</td>
<td>1.9 (0.8)</td>
<td>0.3-3.5</td>
<td>0.5, 3.5</td>
</tr>
</tbody>
</table>

SD = standard deviation.

M-mode measurements: IVSd, interventricular septal thickness in diastole; IVSs, interventricular septal thickness in systole; LVIDd, left ventricular internal diameter in diastole; LVIDs, left ventricular internal diameter in systole; LVFWd, left ventricular free wall thickness in diastole; LVFWs, left ventricular free wall thickness in systole.
4.3 New allometric scaling coefficients

The new proportionality constants (a’) and the scaling exponents (b’) of the AS equation $Y = a'M^b$ were calculated based on the measured echocardiographic values in our Dachshunds (Table 2). The new proportionality constants (a’) derived from this study were comparable with the proportionality constants (a) derived from the previous multi canine breed study. However, the values of the new scaling exponents (b’) derived from this study showed a wider range (0.129 to 0.397) compared to the values of the previous scaling exponents (b) (0.222 to 0.315) reported in the previous study and was less consistent with the presumed index of body length in the allometric equation, which is logarithmic BW raised to 1/3 power.

Table 2. Allometric equation coefficients for the equation $Y = aM^b$ for the estimation of normal M-mode measurements (cm).

<table>
<thead>
<tr>
<th>Measurement (mm)</th>
<th>Current study</th>
<th>Previous study$^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a’</td>
<td>b’</td>
</tr>
<tr>
<td>IVSd</td>
<td>0.461</td>
<td>0.148</td>
</tr>
<tr>
<td>IVSS</td>
<td>0.659</td>
<td>0.129</td>
</tr>
<tr>
<td>LVIDd</td>
<td>1.441</td>
<td>0.306</td>
</tr>
<tr>
<td>LVIDs</td>
<td>0.694</td>
<td>0.397</td>
</tr>
<tr>
<td>LVFWd</td>
<td>0.399</td>
<td>0.239</td>
</tr>
<tr>
<td>LVFWs</td>
<td>0.658</td>
<td>0.189</td>
</tr>
</tbody>
</table>

Allometric scaling equation ($Y = aM^b$ or $Y = a'M^b$): $Y$ represents a measure of heart size, $M$ is bodyweight, $a$ is the proportionality constant, $b$ is the scaling exponent, $a'$ is the new proportionality constant, $b'$ is the new scaling exponent.

M-mode measurements: IVSd, interventricular septal thickness in diastole; IVSS, interventricular septal thickness in systole; LVIDd, left ventricular internal diameter in diastole; LVIDs, left ventricular internal diameter in systole; LVFWd, left ventricular free wall thickness in diastole; LVFWs, left ventricular free wall thickness in systole.

4.4 Reconstruction of normal left ventricular M-mode prediction intervals for Dachshunds

The predicted M-mode values for Dachshunds with variable BW together with their 95% prediction interval were calculated based on the newly established coefficients (a’) and (b’) are tabulated in Table 3. For example, the LVIDd for an 8 kg Dachshund = 1.441 x 80.306 = 2.72 cm (27.2 mm). Both the values of the upper and lower limits of the normal range of LVIDs established in this study were consistently 2 to 3 mm lower than the values predicted by the previous multi canine breed AS study⁶. Except for LVIDd and LVIDs, the width of the prediction intervals for the rest of the M-mode parameters in this study were narrower than the previously described AS prediction intervals.⁶

4.5 Validity of new allometric scaling constants for the prediction of normal M-mode measurements in Dachshunds

Five M-mode parameters (except for LVIDs) had at least 95% or greater of their measured values falling within the new prediction intervals established using the new AS constants derived from this study (Table 4). Approximately 93% of the measured LVIDs values fell within the new prediction interval. When compared to previous multi canine breed AS study⁶, four of the currently measured M-mode parameters (IVSd, IVSs, LVFWd and LVFWs) had at least 95% and LVIDs had only 83% of their measured values falling within the prediction intervals.

The scatter plot graphs for all measured M-mode parameters (except for LVIDd) showed that the upper limits of prediction interval established by previous multi canine breed AS study⁶ were substantially greater than most of the measured values M-mode values, particularly with increasing BW (Fig. 6A-F).
Table 3. Reconstruction of normal M-mode average values (prediction intervals) for Dachshunds.

<table>
<thead>
<tr>
<th>Weight (kg)</th>
<th>IVSd (mm)</th>
<th>IVSs (mm)</th>
<th>LVIDd (mm)</th>
<th>LVIDs (mm)</th>
<th>LVFWd (mm)</th>
<th>LVFWs (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>5.4 (4.0, 7.3)</td>
<td>7.6 (5.8, 10.0)</td>
<td>20.2 (15.8, 25.7)</td>
<td>10.7 (7.2, 15.9)</td>
<td>5.2 (3.9, 6.9)</td>
<td>8.1 (6.1, 10.8)</td>
</tr>
<tr>
<td>4.0</td>
<td>5.7 (4.3, 7.5)</td>
<td>7.9 (6.1, 10.2)</td>
<td>22.0 (17.6, 27.6)</td>
<td>12.0 (8.4, 17.3)</td>
<td>5.6 (4.2, 7.3)</td>
<td>8.6 (6.6, 11.1)</td>
</tr>
<tr>
<td>5.0</td>
<td>5.9 (4.5, 7.6)</td>
<td>8.1 (6.4, 10.4)</td>
<td>23.6 (19.0, 29.3)</td>
<td>13.2 (9.3, 18.6)</td>
<td>5.9 (4.5, 7.6)</td>
<td>8.9 (6.9, 11.5)</td>
</tr>
<tr>
<td>6.0</td>
<td>6.0 (4.6, 7.8)</td>
<td>8.3 (6.6, 10.5)</td>
<td>24.9 (20.2, 30.8)</td>
<td>14.1 (10.1, 19.8)</td>
<td>6.1 (4.8, 7.9)</td>
<td>9.2 (7.2, 11.8)</td>
</tr>
<tr>
<td>7.0</td>
<td>6.2 (4.8, 7.9)</td>
<td>8.5 (6.7, 10.7)</td>
<td>26.1 (21.2, 32.2)</td>
<td>15.0 (10.8, 21.0)</td>
<td>6.4 (5.0, 8.1)</td>
<td>9.5 (7.5, 12.1)</td>
</tr>
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<td>8.0</td>
<td>6.3 (4.9, 8.1)</td>
<td>8.6 (6.8, 10.9)</td>
<td>27.2 (22.1, 33.5)</td>
<td>15.9 (11.4, 22.1)</td>
<td>6.6 (5.1, 8.4)</td>
<td>9.8 (7.7, 12.4)</td>
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<td>9.0</td>
<td>6.4 (5.0, 8.2)</td>
<td>8.8 (6.9, 11.1)</td>
<td>28.2 (23.0, 34.7)</td>
<td>16.6 (11.9, 23.2)</td>
<td>6.8 (5.3, 8.6)</td>
<td>10.0 (7.8, 12.7)</td>
</tr>
<tr>
<td>10.0</td>
<td>6.5 (5.0, 8.4)</td>
<td>8.9 (7.0, 11.2)</td>
<td>29.2 (23.7, 35.9)</td>
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<td>10.2 (8.0, 13)</td>
</tr>
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<td>11.0</td>
<td>6.6 (5.1, 8.5)</td>
<td>9.0 (7.1, 11.4)</td>
<td>30.0 (24.3, 37.0)</td>
<td>18.0 (12.8, 25.2)</td>
<td>7.1 (5.5, 9.1)</td>
<td>10.4 (8.1, 13.2)</td>
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<tr>
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<td>6.7 (5.1, 8.7)</td>
<td>9.1 (7.2, 11.6)</td>
<td>30.8 (24.9, 38.1)</td>
<td>18.6 (13.2, 26.2)</td>
<td>7.2 (5.6, 9.3)</td>
<td>10.5 (8.2, 13.5)</td>
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<td>13.0</td>
<td>6.7 (5.2, 8.8)</td>
<td>9.2 (7.2, 11.7)</td>
<td>31.6 (25.5, 39.2)</td>
<td>19.2 (13.6, 27.2)</td>
<td>7.4 (5.7, 9.5)</td>
<td>10.7 (8.3, 13.7)</td>
</tr>
<tr>
<td>14.0</td>
<td>6.8 (5.2, 8.9)</td>
<td>9.3 (7.3, 11.9)</td>
<td>32.3 (26.0, 40.2)</td>
<td>19.8 (13.9, 28.1)</td>
<td>7.5 (5.8, 9.7)</td>
<td>10.8 (8.4, 14.0)</td>
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<tr>
<td>15.0</td>
<td>6.9 (5.3, 9)</td>
<td>9.4 (7.3, 12.0)</td>
<td>33.0 (26.5, 41.2)</td>
<td>20.3 (14.3, 29.0)</td>
<td>7.6 (5.9, 9.9)</td>
<td>11.0 (8.5, 14.2)</td>
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</tbody>
</table>

M-mode measurements: IVSd, interventricular septal thickness in diastole; IVSs, interventricular septal thickness in systole; LVIDd, left ventricular internal diameter in diastole; LVIDs, left ventricular internal diameter in systole; LVFWd, left ventricular free wall thickness in diastole; LVFWs, left ventricular free wall thickness in systole.
Table 4. Validity of allometric scaling constants for the prediction of normal M-mode measurements in Dachshunds.

<table>
<thead>
<tr>
<th></th>
<th>Current study</th>
<th>Previous study*</th>
<th>Correlation†</th>
<th>Means‡</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>% Within</td>
<td>% Below</td>
<td>% Above</td>
<td>% Within</td>
</tr>
<tr>
<td>IVSd</td>
<td>98</td>
<td>2</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>IVSs</td>
<td>98</td>
<td>2</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>LVIDd</td>
<td>95</td>
<td>5</td>
<td>0</td>
<td>93</td>
</tr>
<tr>
<td>LVIDs</td>
<td>93</td>
<td>7</td>
<td>0</td>
<td>83</td>
</tr>
<tr>
<td>LVFWd</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100</td>
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<tr>
<td>LVFWs</td>
<td>98</td>
<td>2</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

*Percent of values within and outside the prediction interval calculated using the allometric scaling constants as previous reported6.
†Correlation estimated between measured values and those predicted using the allometric scaling constants previously reported6.
‡Mean difference between measured values subtracting those predicted using the allometric scaling constants previously reported6. P value based on paired t tests.

Measured values of LVIDd, LVIDs, LVFWd and LVFWs in this study had statistically significant positive correlations with the predicted values from the previous multi canine breed AS study6 (P < 0.05). The strength of these positive linear correlations ranged from weak (LVFWs, r = 0.363) to strong (LVIDd, r = 0.617). There were no significant correlations between the measured values of IVSd and IVSs with the predicted values from the previous multi canine breed AS study6.

A consistent and similar linear pattern of bias or discrepancy in the Bland-Altman plots was observed between measured values and predicted values for all six left ventricular M-mode parameters (Fig. 7A-F). The findings from the Bland-Altman plots suggested that the predicted values from previous multi canine breed AS study6 tended to overestimate M-mode values at larger body weights while underestimating in Dachshunds with smaller body weights.
FIG. 6. Scatter plots comparing measured left ventricular M-mode values of Dachshunds with predicted values and prediction intervals established by previous multi canine breed allometric scaling study. Faint dotted line represents the allometric scaling (AS) predicted values and solid lines represent the 95% prediction intervals of the predicted values. IVSd, interventricular septal thickness in diastole (a); IVSs, interventricular septal thickness in systole (b); LVIDd, left ventricular internal diameter in diastole (c); LVIDs, left ventricular internal diameter in systole (d); LVFWd, left ventricular free wall thickness in diastole (e); LVFWs, left ventricular free wall thickness in systole (f).
FIG. 7. Bland-Altman plots showing distribution of bias of measured left ventricular M-mode values of Dachshunds when compared with predicted values established by previous multi canine breed allometric scaling study. Horizontal solid lines represent the 95% confidence interval of the bias (measured value – allometric scaling predicted value). IVSd, interventricular septal thickness in diastole (a); IVSs, interventricular septal thickness in systole (b); LVIDd, left ventricular internal diameter in diastole (c); LVIDs, left ventricular internal diameter in systole (d); LVFWd, left ventricular free wall thickness in diastole (e); LVFWs, left ventricular free wall thickness in systole (f).
4.6 Effect of adding other independent variables to the new allometric scaling model for the prediction of normal left ventricular M-mode measurements

Neutering status and BCS had a significant negative correlation with LVIDd and LVIDs (P < 0.05) when added to the AS equation (Table 5). Age, gender and girth diameter were not significantly correlated with any of the measured M-mode parameters.
Table 5. The effect of adding other independent variables to the allometric scaling model for the prediction of normal M-mode measurements.

<table>
<thead>
<tr>
<th>Variable/measure</th>
<th>IVSd</th>
<th>IVSs</th>
<th>LVIDd</th>
<th>LVIDs</th>
<th>LVPWd</th>
<th>LVPWs</th>
</tr>
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<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coefficient</td>
<td>-0.005</td>
<td>0.010</td>
<td>-0.004</td>
<td>-0.003</td>
<td>0.022</td>
<td>0.017</td>
</tr>
<tr>
<td>( r^2 ) change</td>
<td>0.4</td>
<td>1.9</td>
<td>0.2</td>
<td>0</td>
<td>7.6</td>
<td>4.8</td>
</tr>
<tr>
<td>P-value</td>
<td>0.704</td>
<td>0.391</td>
<td>0.713</td>
<td>0.865</td>
<td>0.055</td>
<td>0.148</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coefficient</td>
<td>-0.009</td>
<td>-0.031</td>
<td>-0.028</td>
<td>-0.074</td>
<td>-0.011</td>
<td>0.012</td>
</tr>
<tr>
<td>( r^2 ) change</td>
<td>0.1</td>
<td>1.7</td>
<td>1.2</td>
<td>3.7</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>P-value</td>
<td>0.826</td>
<td>0.414</td>
<td>0.400</td>
<td>0.164</td>
<td>0.790</td>
<td>0.758</td>
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<tr>
<td><strong>Neutered</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coefficient</td>
<td>0.072</td>
<td>0.066</td>
<td>-0.089</td>
<td>-0.122</td>
<td>0.034</td>
<td>0.047</td>
</tr>
<tr>
<td>( r^2 ) change</td>
<td>6.8</td>
<td>6.7</td>
<td>10.7</td>
<td>8.8</td>
<td>1.4</td>
<td>3.0</td>
</tr>
<tr>
<td>P-value</td>
<td>0.093</td>
<td>0.096</td>
<td>0.008*</td>
<td>0.028*</td>
<td>0.421</td>
<td>0.251</td>
</tr>
<tr>
<td><strong>Body condition</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Coefficient</td>
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<td>0.063</td>
<td>-0.060</td>
<td>-0.093</td>
<td>0.030</td>
<td>0.065</td>
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<tr>
<td>( r^2 ) change</td>
<td>6.8</td>
<td>6.7</td>
<td>10.7</td>
<td>8.8</td>
<td>1.4</td>
<td>3.0</td>
</tr>
<tr>
<td>P-value</td>
<td>0.110</td>
<td>0.055</td>
<td>0.039*</td>
<td>0.048*</td>
<td>0.397</td>
<td>0.057</td>
</tr>
<tr>
<td><strong>Girth</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Coefficient</td>
<td>0.017</td>
<td>0.011</td>
<td>0.001</td>
<td>-0.006</td>
<td>-0.008</td>
<td>-0.009</td>
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<tr>
<td>( r^2 ) change</td>
<td>6.3</td>
<td>3.1</td>
<td>0</td>
<td>0.3</td>
<td>1.2</td>
<td>1.9</td>
</tr>
<tr>
<td>P-value</td>
<td>0.107</td>
<td>0.265</td>
<td>0.869</td>
<td>0.678</td>
<td>0.454</td>
<td>0.363</td>
</tr>
</tbody>
</table>

M-mode measurements: IVSd, interventricular septal thickness in diastole; IVSs, interventricular septal thickness in systole; LVIDd, left ventricular internal diameter in diastole; LVIDs, left ventricular internal diameter in systole; LVFWd, left ventricular free wall thickness in diastole; LVFWs, left ventricular free wall thickness in systole.

*Significant negative correlation.
Chapter 5: DISCUSSION

5.1 Introduction

Two-dimensional LA/Ao ratios and left ventricular M-mode transthoracic echocardiographic measurements in 40 clinically normal adult Dachshunds were obtained. Prevalence of MVP in this study was also calculated. This section compares the cut-off values and prediction intervals obtained with the prediction intervals of previous studies and discusses the reasons for similarities or variations observed. The limitation of this study as well as the clinical applications and future research avenues are also discussed.

5.2 Prevalence of mitral valve prolapse in Dachshunds

The prevalence of MVP > 0 mm in this study was 45%, of which 27% had MVP > 1 mm. This value is similar to the 47% prevalence of MVP in Dachshunds reported in one previous study while a second study recorded a much higher prevalence of 81%.\textsuperscript{17,18} The latter study may have had a significantly higher prevalence of MVP because of the inclusion of 18 families of Dachshunds (consisting of both parents with 4 or more offspring) that inherently increased the likelihood of MVP inheritance. Although the number of dogs used in our study was less, they were from diverse origins with reduced chance of a familial relationship amongst each other. Additionally, our study had a larger proportion of smooth-haired Dachshunds (65%) and less long-haired Dachshunds (27%) and wire-haired Dachshunds (7%) compared to the previous second study\textsuperscript{17} in which the majority were long-haired Dachshunds (48%) and wire-haired Dachshunds (41%) with only a small proportion of smooth-haired Dachshunds (11%).

5.3 2-D left atrium to aorta ratio

The maximum upper limits (mean + 2SD) of the LA/Ao ratio for all three methods: the diameter (LA/Ao ratio = 1.66), circumference (LA/Ao ratio = 2.53) and cross-sectional...
area (LA/AO ratio = 3.91) methods derived from our study were very similar to the upper limits established by a previous study. The previous study involved 36 breeds (only one intact male Dachshund was included) with majority of the dogs weighing between 10-40 kg, had the maximum upper limit (mean + 2SD) of the LA/Ao ratio of 1.59, 2.45 and 3.85 for the three methods respectively. Although the BW range of our Dachshunds (5-12.6 kg) was similar to the BW range in a CKCS study (5.5-11.9 kg), the cut-off values of the LA/Ao ratio of Dachshunds and CKCS differed markedly. In our study, 100% of the Dachshunds had LA/Ao ratio < 1.67 (diameter method) which differed markedly from the LA/Ao ratio < 1.28 for the CKCS study. As the value of LA/Ao ratio is derived from two linear dimensions, the ratio would be expected to remain constant in animals that are geometrically similar but differ in terms of body size. The marked difference in LA/Ao ratio between Dachshunds and CKCS is because Dachshunds are chondrodysplastic dwarfs with normal trunk and short legs. Therefore, specific LA/Ao ratios should be established for different canine breeds with different geometrical conformation. Our study however did not include any Dachshund with clinical MMVD. Hence, we are uncertain of the proportion of Dachshunds with clinical MMVD that may have LA/Ao ratio lower than the upper limits established in this study (eg: a normal Dachshund with LA/Ao ratio of 1.3 may progressed to 1.6 if having clinical MMVD and may still be lower than cut-off ratio of 1.67). The M-mode method of measuring LA/Ao ratio was not included in this study due to its inherent limitations, including the difficulty in acquiring the maximum aortic diameter and measuring the auricle rather than the atrium.

5.4 Scaling exponent of the allometric scaling equation

The deviation of the values of the new scaling exponent, (b’ values of 0.129 to 0.397) from the presumed index of body length in allometric equation proposed by the previous multi canine breed study, which is 1/3, may possibly be due to the non-quantifiable effect of thoracic conformation (e.g. Dachshunds being chondrodysplastic dwarfs) and breed specificity. Other possible explanations include the small sample size in this study and more narrow range of BW of the Dachshunds.
5.5 **Comparison of left ventricular M-mode prediction intervals established from this study with prediction intervals established by previous multi canine breed study**

Four of the six left ventricular M-mode parameters had narrower prediction intervals than those predicted by the previous multi canine breed AS study and there was an increased percentage of measured values falling within the new prediction intervals. In particular, only 7% of measured values of LVIDs fell below our current prediction intervals compared to 17% of measured values of LVIDs falling below the prediction intervals of previous multi canine breed study. This suggests that the current prediction intervals established in our study are more representative for Dachshunds than previously derived prediction intervals from the multi canine breed AS study. Except for LVIDd, the upper limits of the new prediction intervals were all lower than the upper limits of the previous multi canine breed AS study. The skewed upper limits of the previous multi canine breed AS prediction intervals may have been due to normalization of values of dogs with a wide range of BW (from 2 kg to 95 kg) and wide variation in body conformation with an over-representation of large breed dogs. We are uncertain if the skewed upper limit of previous multi canine breed AS prediction intervals may have included elevated M-mode values of Dachshunds with subclinical cardiac disease but we believe that there is less likelihood of such inclusion with the more narrow prediction intervals established from our study. However, future investigations using Dachshunds with left ventricular volume overload may aid to investigate the aforementioned uncertainty. Although 95% or greater proportion of measured values of four of six of our M-mode parameters fell within the prediction intervals derived from previous multi canine breed AS study, we speculate that this was most likely due to its wider prediction intervals. This has been similarly observed in another study using Whippets. The wide prediction intervals of the previous multi canine breed AS study could also have been due to its diverse source of data.

The more narrow range of the prediction intervals observed in our study compared to the previous multi canine breed AS study may have been due to the use of AMM.
Accuracy of left ventricular M-mode measurements can be improved by aligning the sampling line perpendicular to the short or long axis of the left ventricle but this can be difficult to achieve using conventional echocardiographic techniques. The use of AMM has been shown to make alignment of this sampling line easier and more precise, subsequently reducing the variability of left ventricular measurements,\textsuperscript{34,35} thereby increasing reproducibility and accuracy of the measurements.\textsuperscript{33} Contrary to this, all data used to establish the previous multi canine breed AS prediction intervals were acquired from conventional M-mode measurements.\textsuperscript{6} Additionally, the retrospective nature of the multi canine breed study\textsuperscript{6} could have resulted in M-mode echocardiographic techniques not being strictly standardized (eg: nine different investigators, sedated dog vs non-sedated dog, etc.).

5.6 Effect of adding independent variables with significant correlation to the allometric scaling equation for the prediction of the left ventricular M-mode parameters

Although several reports found no significant effect of gender on left ventricular M-mode measurements,\textsuperscript{9,36,67} more recent studies identified significant differences between male and female IVSd and LVIDd measurements in Estrela Mountain dogs and LVPWd in dogue de Bordeaux dogs.\textsuperscript{59,60} However, addition of the gender factor to the current AS model did not increase the predictability of left ventricular M-mode measurements in our study. Previous studies have shown a positive correlation between age and normalized left ventricular diameter and wall thickness,\textsuperscript{53,113,114} but addition of age to the AS model did not increase the predictability of the left ventricular dimensions in our adult Dachshunds. No previous study has evaluated the effect of girth diameter on left ventricular M-mode measurements. Adding girth diameter to the AS model did not improve the predictability of measured left ventricular dimensions in our study.

The significant negative correlation of neutering status and BCS with LVIDd and LVIDs were interesting observations that have not been reported previously. Neutered animals and animal with higher BCS tended had lower values relative to intact animals of the
same weight. This may be possibly due to presence of more fat than lean mass in neutered animals and animals with higher BCS. As the total number of neutered dogs in our study was relatively small (33 %), the effect of neutering on M-mode left ventricular dimensions should be interpreted with caution and these findings warrant future investigations.

5.7 Fractional shortening and E point to septal separation

Due to its simplicity in acquisition, FS is the most widely used echocardiographic index of global left ventricular systolic function in veterinary patients. In our study, all clinically normal Dachshunds had a FS within 32–53% which is comparable to previously published non-breed specific values of 27-48% and 30-50%. Since FS is not correlated to BW, the range established in this study may be applied to Dachshunds of all body weights. The maximal initial opening of the anterior mitral valve leaflet (E point) is inversely related to the volume and rate of left atrial emptying and thus left ventricular stroke volume. The EPSS measurement has been used as a practical and easily reproducible clinical index of left ventricular function. Increased EPSS values have been suggested to be indicative of systolic dysfunction or mitral valve stenosis. The upper limit of EPSS at 3.5 mm in our study was markedly lower than the upper limit of 6 mm suggested by a previous study. This may be due to the relative smaller body size of Dachshunds compared to the Beagles and German Shepherd dogs included in the previous study. However, it should be cautioned that no clinically sick dogs with volume overload or systolic dysfunction were included in our study and the previous study, and hence it may not be possible to estimate the overlap between normal and abnormal EPSS values.

5.8 Future studies

The natural succession to this study would be to compare LA/Ao ratio and left ventricular M-mode measurements of Dachshunds with left heart failure with the values established in this study with the objective to assess the feasibility of using the current
prediction intervals to differentiate clinically normal adult Dachshunds from Dachshunds with left heart failure.
The following conclusions were deduced from this study:

- The mean (SD) for 2-D LA/Ao ratio measured in 40 clinically normal adult Dachshunds using the diameter, circumference and cross-sectional area methods via RPSA view were 1.40 (0.13), 2.19 (0.17) and 2.95 (0.48) respectively.

- The maximal upper limit for 2-D LA/Ao ratio (mean + 2SD) in 40 clinically normal adult Dachshunds using diameter, circumference and cross-sectional area methods via RPSA view were 1.66, 2.53 and 3.91 respectively, and were very similar to the upper limits established by a previous study\textsuperscript{22}.

- The normal prediction intervals of the left ventricular measurements established via logarithmic transformation and linear regression in this study were found to have more narrow intervals than previous multi canine breed prediction intervals\textsuperscript{6} and were therefore more representative for clinically normal adult Dachshunds.

- The scaling exponents (b’) derived from this study ranged from 0.129 to 0.397 and did not absolutely conform to the presumed index of body length in the allometric equation proposed by the previous multi canine breed study\textsuperscript{6}, which is BW raised to 1/3 power.
REFERENCES


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DO YOU KNOW IF YOUR DACHSHUND HAS MYXOMATOUS MITRAL VALVE DISEASE (MMVD)?

What is MMVD?
MMVD refers to degeneration of the valve of the heart between the left atrium & left ventricle leading to development & progression of left heart failure.

MMVD is the most common heart disease in dogs comprising 75%-80% of canine cardiac disease. Although MMVD is encountered in all dog breeds, the Dachshund is one of the most predisposed breeds.

Cause of MMVD
The exact cause of canine MMVD has not been ascertained but its particular affiliation with breeds such as Dachshund & Cavalier King Charles Spaniel have suggested that genetic factors play a major role. A large scale study involving 190 Dachshunds which also included 31 parents and 92 offspring has indeed found that mitral valve prolapse (an important component in developing MMVD) in Dachshunds is an inherited condition.

Symptoms & Diagnosis
- Early or mild MMVD will be identified by a low-grade heart murmur with no apparent clinical signs.
- Progressive or advance MMVD will exhibit signs of heart failure (eg: exercise intolerance, increased respiratory rate &/or effort & a cough may develop). Syncope (collapse) may also occur after dogs experience abnormal heart rhythms. In some cases sudden death may occur when a catastrophic degree of severity causes the left atrium to rupture.

Diagnosis
- Characteristic murmurs or other telltale symptoms
- Thoracic radiographs
- Electrocardiography (ECG)
- Echocardiography (ultrasound of the heart)

Treatment
- Fortunately, the majority of patients with MMVD do not require any treatment until they show symptoms and most that do tend to live well with drug therapy alone.
- A combination drug therapy protocol, including diuretics, ACE inhibitors, inodilators such as pimobendan & others depending on the specific individual’s needs & stage of disease, is commonly used to control heart failure. Low-sodium diets & exercise...
restriction are also commonly incorporated as adjunctive therapy. Only the severely diseased tend to succumb, in spite of treatment.
-Surgical treatment of mitral valve disease is unlikely to be practically or economically feasible8-10.

Prevention
-Preventing MMVD requires removing affected dogs from the breeding pool. Otherwise, there is no known preventative approach. This is especially critical for extremely predisposed breeds such as the Cavalier King Charles Spaniel & Dachshund.
-Breeding programs aimed at reducing prevalence of MMVD in these breeds have been launched in Europe & North America whereby detailed heart auscultation & echocardiography are used for screening before they enter any breeding program.

South Africa scenario
-There is currently no screening program available for Dachshunds in South Africa even though it is a popular breed locally.
-We at Onderstepoort Veterinary Academic Hospital (OVAH) are currently conducting a study to:
  I. establish normal echocardiographic reference values for Dachshunds
  II. establish normal reference vertebral heart size (VHS) for Dachshunds
  III. establish a normal trachea diameter/thoracic inlet ratio for Dachshunds
-This information is not available at present but is extremely vital to accurately assess the cardiorespiratory function in Dachshunds.

Invitation
We welcome all Dachshund owners & breeders to participate in this study.
-Any Dachshund between 1 to 7 years of age, either sex with no previous history of cardiorespiratory disorder & not of toy size according to Federation Cynologique Internationale (FCI) is suitable.

What is in this study?
-All participating Dachshunds will undergo preliminary screenings that include physical examination, cardiac auscultation & cursory echocardiographic examination at NO COST TO THE OWNER.
-If your Dachshund appears to have no MMVD on preliminary examination, he/she will be selected for a repeat comprehensive echocardiography, ECG, thoracic radiographs, blood pressure measurements & complete blood count at NO COST TO THE OWNER. A SCHEDULED APPOINTMENT will be made according to your convenience to perform all the above tests.
-If your Dachshund is diagnosed with MMVD during preliminary screening echocardiography, a comprehensive echocardiographic examination will still be performed to assess the severity of the condition and a report will be provided at NO COST TO THE OWNER. If the condition is deemed severe, you are advised to consult your veterinarian or OVAH’s veterinarian for appropriate cardiac medication and disease management - THIS HOWEVER WILL BE AT THE OWNER’S OWN COSTS.

**Anonymity of patient information will be maintained in the interest of owners.
If you are interested or have any further queries, kindly contact:
1) Dr CK Lim (cheekin.lim@up.ac.za) Tel: 012-529 8108
2) Prof Robert M. Kirberger (Robert.kirberger@up.ac.za)
References


3. Pedersen HD, Olsen LH, Mow T, Christensen NJ 1999 Neuroendocrine changes in dachshunds with mitral valve prolapse examined under different study conditions. Research in Veterinary Science 66:11-17


# APPENDIX B

## ANIMAL DATA (DACHSHUND 1)

<table>
<thead>
<tr>
<th>Owner:</th>
<th>Name &amp; ID:</th>
</tr>
</thead>
<tbody>
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</table>

<table>
<thead>
<tr>
<th>Date of birth:</th>
<th>Sex:</th>
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<tbody>
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</tbody>
</table>

### Physical examination

Date & time performed:________________

- Body weight:     BSA:
- Habitus:     BCS:
- Rectal temperature:               
- Pulse:               
- Respiration:               
- Hydration status:               
- Mucous membrane:               
  - Heart sound:               
  - Heart rate (bpm):               
  - Murmur grade (I-VI), if present:               

- History of cardiac problem: Yes/No               
  State problem if yes:_________________________

### Quick echo

- Mitral valve- Normal /Abnormal               
- Mitral regurgitation from left apical 4-chamber view (Colour Doppler)– Yes/No               
  If MR present, RJA/LAA = ___________
- Other abnormalities (if present) :

### Haematology

Date & Time performed:________________

- CBC result: Normal/Abnormal               
- Remarks (if abnormal):
  ______________________________________

- Peripheral blood smear:
  ______________________________________
Diagram of the method used to assess the severity of mitral valve prolapse. The hinge points of the two mitral valve leaflets were used to define the plane of the mitral annulus. The maximal degree of protrusion of any point of the mitral leaflets (including the anterior leaflet, posterior leaflet and coaptation point) into the left atrium during systole (with aid of cine-loop) were measured relative to the annulus plane. Leaflets that did not reach the annulus plane were given negative values. The MVP severity was defined as the average of three measurements ($\leq 0\text{mm} = \text{no MVP}; > 0\text{mm but } \leq 1\text{mm} = \text{mild MVP}; > 1\text{mm} = \text{moderate to severe MVP}$).

Oscillometric blood pressure measurements for Dachshunds

ANIMAL DATA (DACHSHUND 1)

<table>
<thead>
<tr>
<th>Owner:</th>
<th>Name/ID:</th>
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<tbody>
<tr>
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</table>

<table>
<thead>
<tr>
<th>Date of birth:</th>
<th>Sex:</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

Date performed:  Time:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Measurement</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Systolic B.P (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic B.P (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure (MAP)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ECG findings:

______________________________________________________________________
______________________________________________________________________
______________________________________________________________________
APPENDIX E

2-D echocardiographic measurements for Dachshund dogs

ANIMAL DATA (DACHSHUND 1)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Measurement (Diameter in mm)</th>
<th>Mean</th>
<th>Measurement (Circumference mm)</th>
<th>Mean</th>
<th>Measurement (Cross-sectional area in mm²)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>MV anterior septal leaflet thickness (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MV posterior septal leaflet thickness (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ao (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA/Ao</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
**APPENDIX F**

M-mode echocardiographic measurements for Dachshund dogs

**ANIMAL DATA (DACHSHUND 1)**

<table>
<thead>
<tr>
<th>Owner:</th>
<th>Name/ID:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of birth:</td>
<td>Sex:</td>
</tr>
</tbody>
</table>

**Date & time performed:**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Measurement</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

- IVSd (cm)
- IVSs (cm)
- LVIDd (cm)
- LVIDs (cm)
- LVPWd (cm)
- LVPWs (cm)
- FS (%)    
- EF (%)    
- EPSS (mm)
PET OWNER CONSENT FORM:

Dear Pet Owner,

Dachshund is known to be predisposed to myxomatous mitral valvular disease (MMVD) (see attached brochure) and we believed that you really care for your pet!

We would like to invite your pet _______________ to participate in our study to:

i) Establish normal echocardiographic reference values for Dachshunds
ii) Establish normal reference vertebral heart size (VHS) for Dachshunds
iii) Establish normal ratio of trachea diameter to other structures seen on lateral thoracic radiographs such as the thoracic inlet, 3rd rib and 4th thoracic vertebra for Dachshunds

To achieve the above objectives we would like to perform some diagnostic procedures for your pet:

A. PHYSICAL EXAMINATION (NO ADDITIONAL COST)
   - Focusing on cardiac and respiratory system

B. SCREENING ECHOCARDIOGRAPHY (NO ADDITIONAL COST)
   - Ultrasound of the heart to screen your pet for myxomatous mitral valve disease
   The above-mentioned procedure is brief, quick, non-invasive and will be performed with your pet conscious
   - If NO EVIDENCE of myxomatous mitral valve disease is detected on the screening echocardiography, your pet will be eligible for further complete diagnostic imaging, ECG & haematology procedures at NO ADDITIONAL COST TO YOU. An appointment will be made the following procedures as these procedures are more time-consuming & your pet will require additional attention:
C. THORACIC RADIOGRAPHS (3 views) under sedation to assess heart size and respiratory system as well as to exclude Spirocerosis (NO ADDITIONAL COST)

D. COMPLETE BLOOD COUNT & BLOOD SMEAR to exclude systemic infection and blood parasite (NO ADDITIONAL COST)

E. ECG ASSESSMENT & COMPLETE ECHOCARDIOGRAPHY to exclude other possible cardiac diseases & establish normal range for the breed Dachshund whenever possible (NO ADDITIONAL COST)

- If your pet is **DIAGNOSED WITH** myxomatous mitral valve disease, a **COMPLEMENTARY** complete echocardiography will be performed to ascertain his/her cardiac function and severity of the condition. A complete echocardiographic report of your pet will be available within 1 week of the procedure.

- If the MMVD is **MILD**, you are recommended to bring your pet for follow up echocardiography every 2 years. **Unfortunately, this will be at the owners’ expense.**

- If the MMVD is **MODERATE TO SEVERE**, you are advised to consult a veterinarian for to tailor for appropriate medical therapy. **Unfortunately, this will be at the owners’ expense.**

* A nominal ZAR 45 will be chargeable for registration of your pet into our hospital system.

I, ______________________________________________________________________ hereby give permission for the procedures mentioned in A & B above to screen for MMVD & will give permission for procedures C, D & E to be performed on my pet (on AN APPOINTED DATE WITH THE CLINICIAN) if no evidence of MMVD is detected in procedures A & B. I fully understood the non-invasive nature & benefits of the above mentioned diagnostic procedures that will be performed on my pet.

Initial: _____________________________________________

Owner signature:____________________________     Date:________________________

Duty Clinician & Signature:_________________________