M-MODE, TWO-DIMENSIONAL, SPECTRAL DOPPLER AND TISSUE DOPPLER ECHOCARDIOGRAPHIC FINDINGS IN 40 HEALTHY PET RABBITS

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ABSTRACT

Next to dogs and cats, rabbits are popular companion animals in the United Kingdom with 1.7 million kept as pets. Almost 60% of these rabbits are registered with a veterinary practice. The number of reported or anecdotal reports describing congenital or acquired cardiac diseases is increasing, which results in a growing demand to perform echocardiography in pet rabbits as clinical cases. Reports of normal echocardiographic findings for comparison with potentially diseased rabbits are very scarce, and most involve the description of selected echocardiographic parameters in small cohorts of young rabbits in a research setting and under the influence of anaesthetic agents.

The aim of the present study was to report the findings of m-mode, two-dimensional, spectral Doppler and tissue Doppler echocardiography in a large cohort of healthy, manually restrained, adult pet rabbits. The feasibility of performing echocardiography was evaluated and normal values were obtained. The correlation of the different echocardiographic measurements with variables such as weight, heart rate and age was analysed. Furthermore, statistical differences of echocardiographic variables between different breeds and sexes were assessed. Ultimately, we aimed to report the prevalence of trivial valvular regurgitation in the same cohort of healthy rabbits.

Forty healthy pet rabbits of breeds commonly found as pets (22 Dwarf Lops, 14 French Lops and 4 Alaskan) underwent a full physical examination and conscious m-mode, two-dimensional, spectral Doppler and tissue Doppler echocardiography. The

median age of the rabbits was 21.5 months and the median weight was 2.9 kg (Dwarf Lops 2.4 kg/ Alaskans 4.35 kg/ French Lops 6.0 kg). Echocardiography with ECG monitoring was feasible in all rabbits and adequate standard echocardiographic and radial colour views and traces could be obtained. However, pulsed-wave tissue Doppler traces of satisfactory quality were only obtained in 85% of cases.

Left atrial and ventricular dimensions were significantly larger in French Lops in comparison to Dwarf Lops and an overall positive correlation with weight was present. No significant differences between breeds were identified for flow velocities. Comparison between breeds was not possible for diastolic waves both for spectral Doppler and tissue Doppler because of low numbers due to division into two groups: separated and summated diastolic waves. Separation of mitral early and late diastolic waves was observed in 40% of cases using conventional spectral Doppler and in > 60% of cases using pulsed-wave tissue Doppler. Most systolic tissue Doppler parameters were significantly higher in French Lops than in Dwarf Lops and/or were positively correlated with weight. Trivial regurgitations were detected at the aortic valve in 7/40 (17.5%) rabbits, at the tricuspid valve in 5/40 (12.5%) and at the pulmonic valve in 1/40 (2.5%) rabbits.

Performing ECG-gated m-mode, two-dimensional, spectral Doppler and tissue Doppler echocardiography is feasible in healthy manually restrained pet rabbits. The results of this study can be used for comparison with echocardiography obtained from clinical cases. Breed specific values should be used when measuring left atrial and ventricular sizes and radial tissue Doppler systolic parameters in French Lops and Dwarf Lops. Moreover, these breed-specific values might also be applicable for rabbits of similar weight. Most other parameters were independent of breed, weight, age, sex and heart rate and can therefore be used for the general pet rabbit population. Pulsed-wave tissue Doppler might be useful for the assessment of diastolic function in rabbits, as a separation of mitral tissue Doppler early and late diastolic waves persist at heart rates resulting in summation of mitral inflow E and A waves. Trivial tricuspid, aortic and pulmonic valvular regurgitations were detected in healthy pet rabbits, but were uncommon.

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ABBREVIATIONS

A	Spectral Doppler mitral late diastolic wave
Aa	Pulsed-wave tissue Doppler late diastolic wave
Al	Alaska/s
Am_{endo}	Radial colour tissue Doppler subendocardial late diastolic wave
Am_{epi}	Radial colour tissue Doppler subepicardial late diastolic wave
Am_t	Radial colour tissue Doppler mid-ventricular late diastolic wave
At	Spectral Doppler tricuspid late diastolic wave
Ao	End-diastolic aortic diameter in short axis
AV	Peak aortic velocity
bpm	Beats per minute
cm	Centimetres
cm/s	Centimetres per second
DL	Dwarf Lop/s
E	Spectral Doppler early mitral diastolic wave

Ea	Pulsed-wave tissue Doppler mitral annulus early diastolic wave
EA	Spectral Doppler summated mitral diastolic waves
EAa	Pulsed-wave tissue Doppler summated mitral annulus diastolic waves
EAm_{endo}	Radial colour tissue Doppler summated subendocardial diastolic waves
EAm_{epi}	Radial colour tissue Doppler summated subepicardial diastolic waves
EAm_{t}	Radial colour tissue Doppler summated midventricular diastolic waves
EAt	Spectral Doppler summated tricuspid diastolic waves
ECG	Electrocardiography
endo	Endocardium
Em_{endo}	Radial colour tissue Doppler subendocardial early diastolic wave
Em_{epi}	Radial colour tissue Doppler subepicardial early diastolic wave
Em_{t}	Radial colour tissue Doppler mid-ventricular early diastolic wave
epi	Epicardium
EPSS	E-point to septal separation
Et	Spectral Doppler tricuspid early diastolic wave

FL	French Lop/s
FS	Fractional shortening
HR	Heart rate
HRA	Individual average heart rate during echocardiography
IQR	Interquartile range
IVRT	Isovolumic relaxation time calculated by spectral Doppler
$IVRT_t$	Isovolumic relaxation time calculated by tissue Doppler
IVSd	Interventricular septal wall thickness in diastole
IVSs	Interventricular septal wall thickness in systole
LAs	Left atrium in end-systole in long axis
LAd	End diastolic left atrial diameter in short axis
LVET	Left ventricular ejection time
msec	Milliseconds
LVDd	Left ventricular internal diameter in diastole
LVDs	Left ventricular internal diameter in systole

LVFWd	Left ventricular free wall in diastole
LVFWs	Left ventricular free wall in systole
MVG	Radial colour tissue Doppler systolic myocardial velocity gradient
PEP	Pre-ejection period
PV	Peak pulmonary flow velocity
Sa	Pulsed-wave tissue Doppler mitral annulus systolic wave
Sm_{endo}	Radial colour tissue Doppler systolic subendocardial wave
Sm_{epi}	Radial colour tissue Doppler systolic subepicardial wave
Sm_{t}	Radial color tissue Doppler systolic mid-ventricular wave
t	Mid-ventricular
TDI	Tissue Doppler imaging
UK	United Kingdom

1. INTRODUCTION

1.1 Rabbit population and echocardiography

The number of rabbits kept as pet animals is generally increasing in certain parts of the world such as the United Kingdom (UK) (Mullan and Main, 2006). It is estimated that there are 1.7 million pet rabbits in the UK (Edgar and Mullan, 2011) and almost 60% of them are registered with a veterinary practice (PDSA, 2011). The level of veterinary involvement with rabbits is growing, this and better husbandry results in a higher average age of rabbits (Mullan and Main, 2006). The likelihood of a general practitioner or a specialist seeing a rabbit suffering from cardiac disease and the necessity of obtaining echocardiography for diagnosis and to assess the severity of the disease is therefore increasing (Reusch, 2006; Pariaut, 2009). Indeed, the numbers of reports describing congenital or acquired cardiac diseases in rabbits (Martin et al., 1987; Marini et al., 1999; Voros et al., 2011; Gava, 2013) and of anecdotally observed cases are growing (Reusch, 2006; Pariaut, 2009).

1.2 M-mode, two-dimensional and spectral Doppler echocardiography

In the English language literature (to the best of the author's knowledge) echocardiographic findings and normal measurements in healthy rabbits have typically been described from young, often male, New Zealand research rabbits (Fontes-Sousa et al., 2006; Stypmann et al., 2007; Fontes-Sousa et al., 2009; Mora et al., 2009; Gava et al., 2013), who in general, are genetically very similar to one another. Additionally, these were usually under the influence of sedatives or anaesthetic agents, which can alter many echocardiographic values (Stypmann et al., 2007). Extrapolating the normal values obtained from those rabbits to pet rabbits undergoing echocardiography as clinical patients has therefore limitations. Furthermore, common pet breeds vary quite widely in conformation and include smaller rabbits such as Dwarf Lops (DL) or Mini Lops and larger rabbits such French Lops (FL), German Lops or Alaskas (Al), which may differ in heart size and echocardiographic values.

1.3 Tissue Doppler imaging echocardiography

In recent years, tissue Doppler imaging (TDI) has emerged as a new method to assess myocardial function and left ventricular filling pressures in humans, small animals and horses (Chetboul et al., 2004a,b; Chetboul et al., 2005a,b; Chetboul et al., 2006a,b; Hori et al., 2007; Yu et al., 2007; Schwarzwald et al., 2009; Decloet, 2013). Conventional two-dimensional and Doppler assessment of systolic and diastolic function are highly influenced by load, which is less of a problem when using TDI (Yu et al., 2007). Three TDI modes are available: pulsed-wave mode, two-dimensional colour mode and m-mode colour mode; which allow specific analysis of radial and longitudinal myocardial motion (Boon, 2011). Radial and longitudinal two-dimensional colour mode and longitudinal pulsed-wave mode are the most commonly used (Yu et al., 2007; Boon, 2011).

To the best of the author's knowledge, the use of radial colour TDI has not been previously reported in rabbits. Furthermore, reports of pulsed-wave mitral annulus TDI were obtained from either young New Zealand research rabbits under the influence of anaesthetic agents or from a transgenic rabbit model (Nagueh et al., 2000; Stypmann et al., 2007; Fontes-Sousa et al., 2009). Anaesthetic or sedative agents can affect systolic and diastolic function and have been shown to alter conventional echocardiographic parameters in rabbits (Stypmann et al., 2007) and TDI values in other species (Chetboul et al., 2004b). Additionally, breed differences for TDI measurements were observed among dogs and cats (Chetboul et al., 2005b; Chetboul et al., 2006a).

1.4 Aims

The aims of this study were:

1) To evaluate the feasibility of performing conscious echocardiographic examinations with simultaneous ECG recording in a large cohort of healthy pet rabbits.

2) To establish normal values for the most commonly used two-dimensional, m-mode, Doppler and tissue Doppler parameters from the same cohort of pet rabbits.

3) To evaluate the breed and sex differences of echocardiographic parameters and correlations with age, weight and heart rate.

4) To establish breed-specific normal values, if differences were detected between breeds.

5) To investigate the prevalence of trivial valvular regurgitations.

2. MATERIAL AND METHODS

2.1 Ethical approval

Ethical approval was granted by the University of Bristol Ethical Review Group.

2.2 Population characteristics and exclusions

Forty-one adult pet rabbits were recruited from pet owners, a local practice or a local breeder. All rabbits had a full clinical history taken and underwent physical examination. Any rabbits with abnormalities on physical examination, evidence of systemic diseases or cardiovascular anomalies (e.g. arrhythmias or murmurs) or judged to be too stressed on physical examination were excluded from the study.

2.3 Specific preparation for echocardiography

Breed, age, sex and weight of every rabbit were recorded. An area of around 0.5 x 0.5 cm was clipped at the right and left dorsal antebrachium and either the right or left dorsal metarsal or dorsal tibial area. One ECG pad each was placed on the clipped areas and were held in place by tape for continuous ECG monitoring. For echocardiography both sides of the lateral thorax were clipped over the area of the apex beat. All echocardiographic views and measurements were obtained with continuous ECG monitoring by one trained observer (DCS, the author of this thesis) placing the awake rabbits in right and left lateral recumbency on a standard echocardiographic table. Light manual restraint was performed by two experienced assistants. A General Electric Vivid 7 system and a standard phased-array, variable frequency (4.5-11.5 mHz) transducer was used for echocardiography. Images were first acquired and analysis was performed at a later stage on the same station. Rabbits were classified by subjective levels of stress during echocardiography as relaxed, stressed or very stressed. This was assessed subjectively by evaluation of their demeanour, including resistance to lay on the side, making attempts to rise, or an increase in respiratory depth or rate. Echocardiography was stopped if a rabbit was considered to be very stressed.

2.4 Motoring of heart rate during echocardiography

Heart rate (HR) was measured manually (with a specific software tool) for each echocardiographic loop individually to avoid possible spurious results generated by the machine software. The average of all the HR recorded during the examination of every rabbit was defined as HRA .The maximum and minimum HR were also reported.

2.5 Standard transthoracic m-mode, two-dimensional and spectral Doppler echocardiography

Echocardiographic examination and measurements were obtained following standard protocols as described for small animals elsewhere (Boon, 2011). Three cardiac cycles were measured and the average was calculated for each echocardiographic variable. The following measurements were carried out: on a right parasternal long axis view, end systolic left atrial dimension (LAs); on a right parasternal short axis view, m-mode and two-dimensional measurements of the interventricular septal wall thickness in diastole (IVSd) and systole (IVSs), left ventricular internal diameter in diastole (LVDd) and systole (LVDs), left ventricular free wall in diastole (LVFWd) and systole (LVFWs), fractional shortening (FS), E point to septal separation (EPSS), the ratio of end-diastolic left atrial dimension (LAd) to Aorta (Ao) and pulmonic velocity (PV); on a left sided parasternal apical five-chamber view, peak aortic velocities (AV), pre-ejection period (PEP), left ventricular ejection time (LVET), the ratio PEP/LVET and isovolumic relaxation time (IVRT); and on a left sided parasternal four-chamber view, mitral (mitral inflow E wave [E], A wave [A], or summated EA wave [EA]) and tricuspid (tricuspid E wave [Et], A wave [At] or summated EA wave [EAt]) inflow and the ratios of E/IVRT or EA/IVRT. Please note that the right atrioventricular valve is called tricuspid valve for interspecies consistency but it is indeed bicuspid in rabbits (Reusch, 2006).

The potential presence of valvular regurgitations was investigated using colour flow Doppler. The two-dimensional sector size was minimised as much as possible to improve image quality. Colour gain and tissue priority were modified in order to display good colour filling of the chamber or vessel investigated and to minimise potential artefact generation. The highest pulse repetition frequency possible was used to prevent aliased signals of normal flows. For this study, trivial flow was defined as a small valvular leak that extended less than 0.5 cm from the valves. Any rabbit with mild to severe regurgitations was excluded from the study.

2.6 Tissue Doppler echocardiography

Radial colour TDI and mitral annulus pulsed-wave TDI was performed by the same observer (DCS) following standard protocols as described for small animals elsewhere (Chetboul et al., 2004a; Simson et al., 2007; Boon, 2011).

For longitudinal pulsed-wave mitral annulus TDI a left apical four-chamber view was used and the septal and lateral mitral annular motion was measured placing the cursor next to the mitral valve annulus. Gain and filter settings were adjusted to eliminate background noise and to optimise tissue signal recording. Measurements included lateral and septal peak early diastolic (E_a), late diastolic (A_a) and systolic (S_a) mitral valve annular velocities (Figure 1). Subsequent calculations of lateral and septal E_a/A_a and E/E_a ratios were carried out. For radial colour TDI, a right parasternal short axis view at the level of the papillary muscles just below the mitral valve was used. For each examination, the grey scale receive gain was adjusted to optimise imaging of the left ventricular endocardium and epicardium. Real-time colour Doppler images were superimposed on the grey scale with a frame rate of ≥ 200 frames/s and the gain and velocity range were adjusted to maintain optimal colouring and avoid aliasing artifacts. Two different recording modes were applied, a single 2 x 2 mm sampling window, which was placed mid-ventricular (t), and two 1 x 1mm sampling windows, of which one was placed at the endocardium (endo) and one at the epicardium (epi). For each region the following parameters were recorded: systolic velocity (Sm_t, Sm_{endo}, Sm_{epi},, respectively), Em wave peak velocity (Em_t, Em_{endo}, Em_{epi}, respectively), Am wave peak velocity (Am_t, Am_{endo}, Am_{epi}, respectively), summated Em and Am wave peak velocity (EAm_t, EAm_{endo}, EAm_{epi}, respectively) (Figure 2). Systolic myocardial velocity gradients (MVG) defined as the difference between systolic endocardial and epicardial velocities and Em to Am ratios (Em_t/Am_t, Em_{endo}/Am_{endo} and Em_{epi}/Am_{epi}) were calculated from this data. Isovolumic relaxation time ($IVRT_t$) was measured from radial colour TDI traces using the single gate analysis. For all variables, three cardiac cycles were measured and the average was calculated. Images of inadequate quality in terms of alignment or trace quality were excluded from analysis.

2.7 Statistical analysis

The statistical software program SPSS (IBM) was used for analysis of results obtained. Kolmogorov-Smirnov tests were used to assess normality in all continuous variables (with $P<0.05$ taken as significant). For each such variable, the median (with interquartile range [IQR]/5th-95th percentiles) was calculated. In variables with less than 20 values, percentiles were given as 10th-90th and this was indicated in the text or table. Also, when results were given separately by breeds, they were given in 10th-90th percentiles to allow direct comparison independently of the number of cases per variable. Categorical variables such as breed, sex and subjective stress level during echocardiography were described. Differences in distribution for HRA, weight, age, and echocardiographic variables were compared between breeds (FL vs DL), sex, and degree of stress during echocardiography (relaxed vs stressed/very stressed) using t-test for normally distributed data and Mann-Whitney U test for not normally distributed data. However, only echocardiographic variables with more than 30 cases were included in this analysis. Variables, which were statistically different between FL and DL, were reported separately for both breeds. Pairwise correlations between HRA, weight and age among them, and of HR, weight and age with FS, EPSS, PV, AV and all heart size variables were carried out by Pearson's test, if the variables were normally distributed, and by Spearman's rho test, when one not normally distributed variable was present. Correlation between PEP, ET, PEP/ET ratio, IVRT and selected TDI variables (those with more than 30 cases) with HR at the time of measuring these variables, age and weight was also performed in the same manner. Statistical difference between EA/IVRT and E/IVRT; and IVRT and $IVRT_t$, was assessed using Mann-Whitney U test.

3. RESULTS

3.1 Population characteristics and exclusions

Of the 41 rabbits examined, 1 rabbit did not meet the criteria and was excluded from the study. This rabbit had an intermittent arrhythmia detected on auscultation, which was diagnosed as frequent supraventricular premature complexes on ECG. Previous clinical history and physical examination did not reveal significant abnormalities for the other 40 rabbits. Therefore, none of the 40 rabbits included in the study had abnormalities on cardiovascular examination such as arrhythmias or heart murmurs. One rabbit (an Al) met the criteria of not being stressed during physical examination but became very stressed during echocardiography and the examination was abandoned without obtaining all measurements. A complete echocardiographic examination of good quality with continuous ECG recording was possible in all other rabbits. A technical error occurred during image processing in 4 rabbits; IVRT and mitral inflow spectral Doppler images in 1 rabbit and tricuspid inflow spectral Doppler images in 4 rabbits were erroneously deleted and were not available for measurement.

The study group of 40 rabbits included 22 (55%) DL, 14 (35%) FL and 4 (10%) Al. A total of 18 (45%) of these were male and 22 (55%) were female. During echocardiography 33 of 40 (82.5%) rabbits were considered relaxed, 6 of 40 (15%)

were stressed and 1 of 40 (2.5%) was very stressed. The median age of all rabbits was 21.5 months (IQR 12.2-32.7 months/5th-95th percentiles 6.0-59.9 months) and their median weight was 2.9 kg (IQR 2.4-5.2 kg/5th-95th percentiles 1.9-6.8 kg). There was no difference in age between breeds, but FL were significantly heavier than DL, having a median weight of 6.0 kg (IQR 4.7-6.3 kg/ 10th-90th percentiles 4.4-6.9 kg) and 2.4 kg (IQR 2.1-2.5 kg/10th-90th percentiles 1.9-3.0 kg), respectively. Median weight of Al was 4.3 kg (IQR 3.5-4.9 kg). However, Al were not included in statistical analysis of breed variables due to their low number. There were no significant differences in age or weight between the sexes or the stress state of the animals.

3.2 Heart rate during echocardiography

The median HRA was 210 beats per minute (bpm) (IQR 192-243 bpm/ 5th-95th percentiles 159-277 bpm), and the median maximum HR was 223 bpm (IQR 203-260 bpm, 5th-95th percentiles 182-306 bpm) and the median minimum HR was190 bpm (IQR 172-200 bpm/5th-95th percentiles 132-258 bpm). There was no significant difference in HRA between breeds, sexes or stress states, and no correlation between weight with age or HRA; however, there was a positive correlation between age and HRA ($r_{S=}$0.490; P=0.001).

3.3 Results of m-mode, two-dimensional and spectral Doppler echocardiography

Results of the m-mode, two-dimensional and spectral Doppler echocardiographic measurements are shown in tables 1 and 2. All heart size measurements were positively correlated with weight (table 1) and FLs showed significantly larger heart sizes than DL (table 2). No differences were present between sexes or degree of stress, and no correlation was detected with HRA or age, except for LAs and HRA, which were negatively correlated (r_s=-0.353, P=0.025).

The systolic time intervals, PEP and LVET, could be measured in 39 rabbits. The median HR at the time of collection was 214 bpm (IQR 195-246 bpm/5th-95th percentiles 154-280 bpm) and a negative correlation of PEP (r_s=-0.471;P=0.002) and LVET (r_s=-0.731;P<0.001) with HR, but not of their ratio (PEP/LVET), was observed. No correlation with age or weight or differences between breeds, sexes or degrees of stress was detected.

PV and AV measurements were available in 39 rabbits. Both velocities were positively correlated with weight (table 1) and AV was also associated with age (r_s=-0.393; P =0.013). Comparing breeds, FL had higher PV and AV velocities than DL (table 2). No correlation was present with HR, and there were no differences between sexes or levels of stress.

IVRT measured on standard echocardiography was available in 38 cases and the median HR at the time of collection was 212 bpm (IQR 191-245 bpm; 5^{th}-95^{th} percentiles 163-271 bpm). A positive correlation of IVRT with weight (table 1) and a difference between breeds (table 2) was present. There was no correlation with age or HR and no differences between sexes or levels of stress were detected.

In the majority of cases mitral inflow E and A waves (60%, 23/38) and tricuspid inflow Et and At waves (52.5%, 18/35) were summated. The minimum HR at which mitral EA and tricuspid Et and At waves were summated was 201 bpm and 196 bpm, respectively. On the other hand, the maximum HR at which mitral E and A and tricuspid Et and At waves were still separated was 204 bpm for both. Comparison between the ratios of EA/IVRT and E/IVRT showed significantly higher results for EA/IVRT (P=0.037).

Colour flow Doppler investigation revealed a trivial tricuspid regurgitation in 5/40 (12.5%) of rabbits, a trivial aortic regurgitation in 7/40 (17.5%) rabbits and a trivial pulmonic regurgitation in 1/40 (2.5%) rabbits. Mitral regurgitation was not observed. All valvular regurgitations extended less than 0.25 cm from the closed valves into the appendant chamber.

3.3 Results of tissue Doppler echocardiography

Results for pulsed-wave mitral annulus TDI Doppler measurements are shown in table 3. Adequate traces for TDI measurements were obtained in 34/39 (87.1%)

rabbits for lateral and in 33/39 (84.6%) rabbits for septal annulus movements. TDI Em and Am were separated in 62% for the lateral (21/34) and 63.6% for the septal (21/33) wall. The maximum HR at which TDI Em and Am waves were still separated was 235 bpm for both the septal and lateral wall. The minimum HR at which TDI Em and Am waves summated into EAm was 203 bpm (septal) and 208 bpm (lateral).

Colour radial TDI results are given in table 4. Adequate traces were obtained in all cases. In 21/39 cases (53.8%) Em and Am waves were summated in the regions investigated. The minimum HR at which summated EmAm waves were observed was 195 bpm. The maximum HR at which separation of both waves was detected was 205 bpm.

Comparing both breeds, systolic radial TDI velocities were higher in FL than in DL (Sm_t, P=0.008; Sm_{endo}, P=0.016; Sm_{epi}, P=0.014) (table 5). Higher velocities in FL were also observed for lateral and septal longitudinal Sa, but these did not reach statistical significance. No significant differences in TDI velocities were observed between sexes. Furthermore, $IVRT_t$ and MVG were not significantly different between breeds or sexes.

Four of the systolic parameters were positively correlated with weight (Sm_t r=0.549, P<0.001; Sm_{endo} r=0.448, *P*=0.002; Sm_{epi} r=0.494, *P*=0.001; Sa septal r=0.426, *P*=0.015) and two of them with HR at the time of acquisition (Sm_{endo} *P*=0.016, r=0.383; Sm_{epi} *P*=0.020, r=0.372). No significant correlation with age was detected. $IVRT_t$ and MVG were not significantly correlated with HR at the time of acquisition, weight or age. Also, $IVRT_t$ was not significantly different to IVRT.

4. DISCUSSION

4.1 General overview

The present study is the first report of echocardiographic findings in healthy adult conscious pet rabbits of breeds FL, DL and Al. The median age of the rabbits included was two years with an equal distribution of both sexes. DL and FL were the main breeds, which are two of the most common pet breeds in the UK (Mullan and Main, 2006). The study population reflects therefore a typical rabbit population seen in clinical practice, which makes our results useful as reference for general practitioners, exotic specialists and cardiologists, performing standard m-mode, two-dimensional and spectral Doppler echocardiography in rabbits.

The study reports further the findings of TDI echocardiography in the same cohort of rabbits. TDI is increasingly used in daily practice supporting the diagnosis of various cardiac diseases in cats, dogs and humans (Chetboul et al., 2005a,b; Chetboul et al., 2006a; Boon, 2011). Systolic and diastolic parameters, such as Sm or Sa and Ea or Em, respectively, or predictors of left ventricular filling pressures such as E/Ea, have been shown to be useful to obtain a diagnosis and provide independent prognostic information in people with heart failure, myocardial infarction or hypertensive cardiomyopathy (Yu et al., 2007a). In veterinary medicine, these parameters have been applied for the evaluation of myocardial function and left ventricular filling pressures in dogs with dilated cardiomyopathy or mitral valve disease and in cats with hypertrophic cardiomyopathy (Chetboul et al., 2006b,c; Sampedrano et al., 2006; Chetboul et al., 2007; Hori et al., 2007; MacDonald et al., 2007; Tidholm et al., 2009; Schober et al., 2010). To investigate whether TDI parameters are also beneficial for the assessment of cardiac diseases in rabbits, further studies are required, however, the availability of the normal values reported in this study will allow the comparison to clinical cases in practice.

4.2 Feasibility of performing echocardiography in rabbits

Echocardiography was feasible and uneventful in all but one rabbit. This low complication rate may have been due to the availability of well-trained nurses experienced in rabbit handling, which is fundamental when working with this species. The resulting number of rabbits being stressed is similar to what one may encounter in cats or dogs. Continuous ECG monitoring was easily performed and diagnostic traces were obtained in all rabbits.

4.3 Feasibility of obtaining adequate m-mode, two-dimensional, spectral Doppler and Tissue Doppler images and traces

M-mode, two-dimensional and spectral Doppler images of adequate quality were obtained from all rabbits. Adequate radial systolic and diastolic TDI traces were also adquired from all rabbits. However, longitudinal pulsed-wave TDI of adequate quality was only obtained in 85% of rabbits (33/39 for septal and 34/39 for lateral traces). This might be caused by the requirement of narrow windows, precise alignment and the necessity of obtaining the trace at the time point of the examination (Chetboul et al., 2004a; Simpson et al., 2007; Boon, 2011). Nevertheless, the high percentage of adequate quality traces obtained in our study does encourage the recording of TDI measurements from conscious pet rabbits.

4.4 Heart rate during echocardiographic examination

The HRA of 209 bpm during examination was lower than in two echocardiographic studies, one performed with awake research New Zealand rabbits (238 bpm) (Gava et al., 2013) and one with sedated/anaesthesised research rabbits (258 bpm) (Mora et al., 2009), and higher than in other studies with research rabbits under sedation or anesthesia (155 to 198 bpm) (Fontes-Sousa et al., 2006; Stypmann et al., 2007). Furthermore, this HR was at the lower end for what is reported during physical examination (200-300 bpm) (Pariaut, 2009) and no marked variability among rabbits was observed, with a maximum HR of 223 bpm and a minimum of 190 bpm. This suggests that the rabbits were not markedly affected by the procedure performed.

4.5 Heart size variables and fractional shortening

None of the echocardiographic heart size measurements were correlated with gender and only one (LAs) was moderately correlated with age. However, for every heart size variable we observed significant and robust effects of breed and of weight. These two potential predictor variables are highly correlated ($r_s = 0.852$) and it is therefore difficult to attribute the observed effects to one or the other predictor. This is confirmed by the absence of overlapping of weights identified for DL and FL of the present study. DL are smaller and more compact than FL, which tend to be heavier and bulkier. This is of clinical relevance, as, for example, a normal LVDd for a 6kg FL would be abnormal for a 2kg DL. Therefore, for breed/weight-associated measurements, DL values can be used for DL and breeds of similar size such as Mini Lops, and FL values for FL and similar breeds, such as German Lops. For rabbits of breeds in between both sizes (e.g. 3.5-4.5 kg), the total values and the interquartile range might be preferable. Unfortunately, only four Al, which would have been a breed between FL and DL, could be recruited for this study and normal breed-specific values could not be obtained.

As it is the case in most species (Boon, 2011), the ratio LA/Ao and FS were independent of weight, breed, sex, age or level of stress, which makes them robust clinical tools.

4.6 Pulmonic and aortic velocities

Slightly lower PV than AV values were detected in the rabbits of this study, which is consistent with results reported in most species (Boon, 2011). Breed differences and a positive correlation with weight was present, which has also been reported in dogs (Boon, 2011). However, these differences are small from a clinical point of view and therefore unlikely to be of clinical significance. In contrast to what has been reported in dogs (Boon, 2011), a correlation of AV and PV with HR was not detected in the rabbits. This might be caused by the high and constant HR, which limits the variability of ventricular filling and subsequent stroke volume (Katz, 2011).

4.7 Systolic time intervals, E-point to septal separation and systolic tissue Doppler parameters

PEP, LVET, PEP/LVET and EPSS, which are used for assessment of systolic function (Boon, 2011), were not associated with breed or weight and were therefore reported as overall values. PEP and LVET were strongly associated with HR, whereas their ratio (PEP/LVET) was not. This is also known for other species (Boon, 2011), and favors the use of the ratio over PEP and LVET separately. Systolic TDI waves may also prove useful in the assessment of systolic function. The detected correlation of most systolic TDI measurements with weight and the observed difference in systolic radial color TDI parameters between breeds may be of clinical relevance, and these values were reported separately for each breed. Bigger rabbits (FL) had higher systolic colour Doppler values, which is similar to what is reported in dogs (Chetboul 2005a) and should therefore also be taken into account when evaluating systolic function by TDI in this species. An interesting positive correlation was observed between HR and some systolic TDI values, which suggests an increased sympathetic tone and thereby contractility (Stoylen et al., 2003).

4.8 Diastolic function and filling pressures variables: isovolumic relaxation time, mitral inflow waves, and tissue Doppler diastolic parameters

Early and late mitral inflow Doppler and TDI waves are important echocardiographic tools in the assessment of diastolic function but the flow profile requires separation of both waves for this purpose (Boon, 2011). Unfortunately, with high HR, summation of both waves occurs, which was also detected in the rabbits of the current study. Assessment of diastolic function by mitral valve E and A waves was possible in 40% of rabbits only, because the rapid HR led to summated E and A waves. This might limit the applicability of mitral inflow for the assessment of diastolic function in rabbits. Furthermore, it might have caused that we diagnosed some rabbits with diastolic dysfunction as healthy. However, in some rabbits TDI early and late diastolic waves, and particularly pulse-wave TDI waves, remained separated at HR at which mitral inflow E and A waves were summated. TDI might therefore be a valuable extra tool for assessing diastolic function in rabbits. Diastolic early and late waves measurements both in spectral Doppler or TDI could not be compared between breeds, because separation of summated and separated mitral inflows reduced the numbers of cases in each category and precluded comparisons. As expected and observed in other species (Boon, 2011), summated EA waves were higher than E waves.

E/Ea ratios are used in dogs and people as a predictor of left ventricular filling pressures (Yu et al., 2007; Schober et al., 2010). Measurements of E/Ea in the rabbits were similar to what has been reported for other species (Yu et al., 2007; Schober et al., 2008; Schober et al., 2010; Boon, 2011) and further studies may validate its use in rabbits in clinical practice.

Measuring isovolumic relaxation time (IVRT and $IVRT_t$) is an indirect measurement of ventricular relaxation and commonly used for the assessment of diastolic function

(Boon, 2011). In this cohort of rabbits, IVRT was of great consistency and little variability. In humans, cats and dogs HR is suspected to be negatively correlated with IVRT (Boon, 2011), however, a correlation with HR was not observed in the rabbits of our study. In the same manner, $IVRT_t$ was also not correlated with HR. This absence of correlation with HR which is different to other species (Boon, 2011) may be due to the high HR and low HR variability observed in the rabbits of our study. We also observed no statistical difference between IVRT and $IVRT_t$, which is different to other species (Boon 2011), and both methods could therefore be used interchangeably to measure isovolumic relaxation time in the rabbit. A moderate correlation of IVRT with weight was observed and FL had slightly higher IVRT than DL. This is however of unlikely clinical significance considering the little variability of this parameters and the minimal clinical difference. Nevertheless, IVRT is reported separately for each breed.

As expected, higher summated EA resulted in higher EA/IVRT than E/IVRT. Nevertheless, both ratios could prove useful for assessing left ventricular filling pressures and further studies including rabbits with cardiac diseases are required.

4.9 Trivial valvular insufficiencies

Trivial valvular insufficiencies are described as small valvular leaks without hemodynamic significance (Boon, 2011). These are common in people and have been also extensively reported in other species such as dogs or horses (Choong et al., 1989; Boon, 2011). Although reported prevalences vary among these species, in dogs, pulmonary insufficiencies are the most common, followed by tricuspid, mitral and aortic insufficiencies (Boon, 2011). In the rabbits aortic valve regurgitation was the most common valvular insufficiency, followed by tricuspid and pulmonic insufficiencies. Interestingly, no rabbit was observed to have mitral valve regurgitation.

4.10 Study limitations

This study has several limitations. The rabbits of this study were considered healthy on the basis of an unremarkable clinical history and no abnormalities on physical and cardiovascular examination. Occult disease can therefore not be ruled out. A larger number of rabbits of a wider geographical distribution would have been preferable, in order to establish reference intervals for the general population. However, including representative common pet rabbit breeds provides normal values for breeds relevant in clinical practice. Furthermore, these values can be applied for breeds of similar sizes to FL and DL. A sufficient number of rabbits from a breed of medium size were unfortunately not available, and no normal values can be given. However, overall results and their interquartile range can be used for these animals. Furthermore, the population consisted of adult-young to adult-middle age rabbits and the values obtained might not be applicable for older rabbits.

Unfortunately, coefficients of inter- and intra-observer variations of the echocardiographic examinations were not obtained, because multiple investigations and re-examinations of rabbits were not possible on ethical grounds; and a representative number of trained observers was not available at our institution.

The multiplicity of statistical tests carried out might have increased the risk of type I errors as positive results may be found simply by chance. Many of the differences and correlations have however P values far lower than 0.05 and would be robust to a lower cut off criterion so we choose to simply report all P values <0.05 and critically review the results. Results with P values above 0.01 and unclear explanation or clinical significance might indeed have been false positives

For the assessment of valvular regurgitations, we used strict criteria. Clear guidelines of what is considered a trivial regurgitation in regards to timing and description of the jet are not available for rabbits, dogs or cats. In humans, insignificant regurgitant jets do not extend more than 1 cm from the closed valve (Choong et al., 1989; Boon, 2011). We therefore, adapted the human criteria and elected to exclude any case with extension of a regurgitant jet more than 0.5 cm from the valve. Indeed, in all rabbits with regurgitations, the regurgitant jet was smaller than 0.25 cm.

4.11 Conclusions

Performing conventional m-mode, two-dimensional, spectral Doppler and TDI echocardiography with continuous ECG monitoring is feasible in manually restrained pet rabbits. We report the echocardiographic findings in 40 healthy adult pet rabbits of breeds commonly seen in practice (DL, FL, Al), which can be used as normal values for comparison with clinical cases. Heart size variables such as luminal dimensions and left ventricular wall thickening were breed and weight dependent with FL having significantly bigger hearts than DL. The majority of systolic TDI parameters were also breed and weight dependent with FL and bigger rabbits having higher values. Most other echocardiographic parameters were independent of breed, weight, age, sex, HR, and level of stress during echocardiography and are therefore applicable for the general adult pet rabbit population. Pulsed-wave TDI might be useful for the assessment of diastolic function in rabbits, as a separation of mitral TDI early and late diastolic waves persists at HR resulting in summation of mitral inflow E and A waves. Trivial tricuspid, aortic and pulmonic valvular regurgitations were observed in healthy pet rabbits, but were uncommon.

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6. APPENDICES

6.1 Table 1

Values for m-mode, two-dimensional, and spectral Doppler echocardiographic parameters obtained from conscious healthy pet rabbits.

For variables that correlated significantly with weight (P<0.05), the level of correlation and significance is additionally shown.

At=tricuspid A wave, Ao=end-diastolic aortic diameter, AV=peak aortic velocity, bpm= beats per minute, E=mitral E wave, EA=summated mitral EA wave, EAt=summated tricuspid EA wave, EPSS=E-point to septal separation, Et=tricuspid E wave, FS=fractional shortening, IVRT=isovolumic relaxation time, IVSd=interventricular septal wall thickness in diastole, IQR= interquartile range, IVSs=interventricular septal wall thickness in systole, LAs=left atrium in end-systole in long axis, LAd=end-diastolic left atrial diameter in short axis, , LVDd=left ventricular internal diameter in diastole, LVDs=left ventricular internal diameter in systole, LVET=left ventricular ejection time, LVFWd=left ventricular free wall in diastole, LVFWs=left ventricular free wall in systole, m=metres, mm=millimetres, msec=milliseconds, PEP=pre-ejection period, PV=peak pulmonary flow velocity, sec=seconds, 2D=two dimensional.

[a]n=39 for PEP, LVET, PV, AV and PEP/LVET, n=38 for IVRT, n=23 for EA and EA/IVRT, n=18 for EAt. n=17 for Et and At, n=15 for E, A and E/IVRT.

[b]Percentiles are 10^{th}-90^{th}.

	All rabbits (n=40)[a]		Variables that correlated significantly with weight
Variable	**Median (IQR)**	**Percentiles 5^{th}-95th**	
Age (months)	21.5 (12.2-32.7)	6.0-59.9	
Weight (kg)	2.9 (2.4-5.2)	1.9-6.8	Not applicable
Heart rate mean (bpm)	209.5 (192-242.5)	158.5-277.1	
LAs (mm)	12.0 (11.0-13.4)	9.6-14.3	r_s=0.587; P<0.001
LAd (mm)	9.4 (8.0-12.0)	6.8-13.3	r_s=0.797; P<0.001
Ao (mm)	7.5 (6.1-8.9)	5.7-8.9	r_s=0.811; P<0.001
LAd/Ao ratio	1.30 (1.22-1.39)	1.10-1.48	
IVSd 2D (mm)	2.5 (2.2-3.1)	1.9-4.0	r_s=0.759; P<0.001
LVDd 2D (mm)	15.2 (13.7-17.8)	11.4-19.7	r_s=0.663; P <0.001
LVFWd 2D (mm)	2.5 (2.3-3.1)	1.8-3.7	r_s=0.692; P <0.001

	All rabbits (n=40)[a]		Variables that correlated significantly with weight
Variable	**Median (IQR)**	**Percentiles 5th-95th**	
IVSs 2D (mm)	3.5 (3.1-3.9)	2.4-4.6	r_s=0.612; P <0.001
LVDs 2D (mm)	9.7 (8.8-11)	6.4-13.0	r_s=0.443; P=0.004
LVFWs 2D (mm)	4.6 (3.6-6.0)	3.0-7.4	r_s=0.744; P <0.001
IVSd M-mode (mm)	2.7 (2.3-3.0)	1.8-3.8	r_s=0.774; P <0.001
LVDd M-mode (mm)	14.8 (12.8-17.3)	11.1-19.2	r_s=0.673; P<0.001
LVFWd M-mode (mm)	2.9 (2.6-3.5)	2.1-4.6	r_s=0.771; P <0.001
IVSs M-mode (mm)	3.7 (3.3-4.5)	2.9-5.9	r_s=0.578; P<0.001
LVDs M-mode (mm)	9.76 (8.8-11.1)	7.3-12.2	r_s=0.598; P<0.001
LVFWs M-mode (mm)	4.2 (3.6-5.4)	3.2-6.8	r_s=0.749; P <0.001
FS 2D (percentage)	35 (32.2-39.7)	30-46	

	All rabbits (n=40)[a]		Variables that correlated significantly with weight
Variable	**Median (IQR)**	**Percentiles 5th-95th**	
FS M-mode (percentage)	36.5 (32.2-39)	30-42.9	
EPSS (mm)	1.3 (1.2-1.3)	0.4-2.7	
PV (In m/sec) n=39	0.80 (0.73-0.94)	0.63-1.14	r_s=0.528; P =0.001
AV (In m/sec) n=39	0.89 (0.76-1.02)	0.57-1.26	r_s=0.519; P =0.001
IVRT (msec) n=38	44 (41-46)	40-48	r_s=0.417; P =0.008
E wave (cm/sec) n=15	61.5 (55-74.7)	49.4-80.8[b]	
A wave (cm/sec) n=15	48.5 (43.0-58.7)	38.5-68.1[b]	
EA wave (cm/sec) n=23	75.4 (64.1-86.2)	45.5.-103.0[b]	
E/IVRT N=15	1.48 (1.01-1.67)	1.13-1.89[b]	
EA/IVRT N=23	1.70 (1.50-1.96)	0.94-2.34[b]	

	All rabbits (n=40)[a]		Variables that correlated significantly with weight
Variable	**Median (IQR)**	**Percentiles 5th-95th**	
Et wave (cm/sec) n=17	46.0 (39.5-49.5)	26.4-55.6[b]	
At wave (cm/sec) n=17	27.0 (24.1-32.1)	24.0-44.2[b]	
EAt wave (cm/sec) n=18	53.5 (44.7-68.5)	40.3-81.2[b]	
PEP (msec) n=39	26 (21-33)	16-37	
LVET (msec) n=39	126 (113-139)	98-153	
PEP/ET N=39	0.22 (0.17-0.26)	0.12-0.34	

6.2 Table 2

Values for m-mode, two-dimensional and spectral Doppler echocardiographic parameters separated by breeds (Dwarf Lops and French Lops).

Ao=end-diastolic aortic diameter, AV=peak aortic velocity, IVRT=isovolumetric relaxation time, IVSd=interventricular septal wall thickness in diastole, IQR= interquartile range, IVSs=interventricular septal wall thickness in systole, LAs=left atrium in end-systole in long axis, LAd=end-diastolic left atrial diamenter in short axis, LVDd=left ventricular internal diameter in diastole, LVDs=left ventricular internal diameter in systole, LVET=left ventricular ejection time, LVFWd=left ventricular free wall in diastole, LVFWs=left ventricular free wall in systole, m=metres, mm=millimetres, msec=milliseconds, PV=peak pulmonary flow velocity, sec=seconds, 2D=two dimensional

	Dwarf Lops (n=22)		French Lops (n=14)		Statistical difference (P value)
Variable	**Median (IQR)**	**Percentiles 10th-90th**	**Median (IQR)**	**Percentiles 10th-90th**	
Weight (kg)	2.4 (2.1-2.5)	1.9-3.0	6.0 (4.7-6.3)	4.4-6.9	P<0.001
LAs (mm)	11.1 (10.1-11.9)	9.6-12.4	13.6 (12.8-14.0)	11.2-14.4	P<0.001
LAd (mm)	8.0 (7.6-8.9)	6.9-9.5	12.4 (11.4-13.0)	11.0-13.7	P<0.001
Ao (mm)	6.2 (5.9-6.6)	5.6-8.0	9.5 (8.7-10.0)	8.0-11.2	P<0.001
IVSd 2D (mm)	2.3 (2.0-2.5)	1.9-2.7	3.3 (2.9-3.7)	2.7-4.0	P<0.001
LVDd 2D (mm)	14.5 (13.3-15.2)	11.8-15.8	18.1 (17.5-18.8)	15.1-20.1	P<0.001
LVFWd 2D (mm)	2.3 (2.0-2.4)	1.8-2.7	3.4 (3.0-3.6)	2.6-4.1	P<0.001
IVSs 2D (mm)	3.2 (2.8-3.4)	2.5-3.9	3.9 (3.7-4.4)	3.6-5.5	P<0.001
LVDs 2D (mm)	9.4 (8.5-10.2)	7.5-10.6	11.2 (9.8-12.05)	5.1-12.8	P=0.001
LVFWs 2D (mm)	3.6 (3.5-4.2)	2.9-4.9	5.8 (5.1-6.6)	4.8-7.5	P<0.001

	Dwarf Lops (n=22)		French Lops (n=14)		Statistical difference (P value)
Variable	**Median (IQR)**	**Percentiles 10^{th}-90^{th}**	**Median (IQR)**	**Percentiles 10^{th}-90th**	
IVSd M-mode (mm)	2.4 (2.1-2.7)	1.8-2.9	3.0 (2.9-3.7)	2.1-4.1	P<0.001
LVDd M-mode (mm)	14.2 (12.3-14.8)	11.1-16.1	18.1 (17.0-18.6)	15.2-19.7	P<0.001
LVFWd M-mode (mm)	2.6 (2.4-2.9)	2.1-3.0	3.9 (3.0-4.4)	2.9-5.3	P<0.001
IVSs M-mode (mm)	3.5 (3.2-3.8)	2.9-4.5	5.0 (4.1-5.6)	3.9-5.9	P<0.001
LVDs M-mode (mm)	9.1 (8.0-10.0)	7.5-10.8	11.3 (10.6-11.9)	9.1-12.6	P<0.001
LVFWs M-mode (mm)	3.7 (3.3-4.0)	3.2-4.2	5.6 (4.9-6.4)	4.6-6.8	P<0.001
PV (In m/sec)	0.75 (0.70-0.85)	0.63-0.93	0.88 (0.73-1.0)	0.70-1.16	P=0.006
AV (In m/sec)	0.76 (0.69-0.83)	0.63-0.93	0.91 (0.74-1.0)	0.73-1.1	P=0.001
IVRT (msec)	42 (40-44)	39-47	46 (44-48)	41-51	P=0.003

6.3 Table 3

Values for longitudinal pulsed-wave tissue Doppler parameters.

Aa=pulse-wave tissue Doppler late diastolic wave, bpm= beats per minute, cm/sec=centimetres per sec, Ea=pulse-wave tissue Doppler early diastolic wave, EAa=pulse-wave tissue Doppler summated diastolic wave, IQR= Interquartile range, Sa=pulse-wave tissue Doppler systolic wave.

[a]Percentiles are 10^{th}-90^{th}.

Variable	Median (IQR)	Percentiles 5th-95th
Sa lateral (cm/sec) n=34	6.95 (6.00-8.52)	4.45-8.82
Ea lateral (cm/sec) n=21	7.90 (7.25-9.82)	5.33-11.43
Aa lateral (cm/sec) n=21	6 (5.35-8.12)	4.11-9.75
EAa lateral (cm/sec) n=13	8.71 (7.35-9.55)	7.11-10.92[a]
Ea/Aa lateral n=21	1.67 (1.17-1.56)	0.75-1.83
E/Ea lateral n=13	7.3 (6.2-10.1)	5.8-11.8[a]
Sa septal (cm/sec) n=33	6.61 (5.15-7.82)	3.85-10.49
Ea septal (cm/sec) n=21	6.40 (6.55-8.4)	4.91-10.14
Aa septal (cm/sec) n=21	6.20 (5.35-7.05)	3.14-10.10
EAa septal (cm/sec) n=12	9.40 (7.42-10.32)	6.2-10.70[a]

Variable	**Median (IQR)**	**Percentiles 5th-95th**
Ea/Aa septal n=21	1.23 (0.95-1.33)	0.72-1.94
E/Ea septal n=14	9.20 (7.81-10.5)	5.67-13.41[a]

6.4 Table 4

Values for radial colour tissue Doppler parameters.

Am_{endo}= subendocardial late diastolic wave, Am_{epi}=subepicardial late diastolic wave, Am_t=mid-ventricular late diastolic wave, cm/sec=centimetres per second, Em_{endo}=subendocardial early diastolic wave, Em_{epi}=subepicardial early diastolic wave, Em_t= mid-ventricular early diastolic wave, EAm_{endo}=summated endocardial diastolic waves, $EAm_{epi=}$summated epicardial diastolic waves, EAm_t=summated mid ventricular diastolic waves, IQR= interquartile range, $IVRT_t$=isovolumic relaxation time, msec=milliseconds, MVG= systolic myocardial velocity gradient, Sm_{endo}=systolic subendocardial wave, Sm_{epi}=systolic subepicardial wave, Sm_t=systolic mid ventricular wave.

[a]Percentiles are 10^{th}-90^{th}.

Variables	Median (IQR)	5^{th}-95^{th} percentiles
Sm_t (cm/sec) n=39	5.44 (4.31-7.76)	3.27-8.45
Em_t (cm/sec) n=18	5.68 (4.51-6.73)	3.23-7.57[a]
Am_t (cm/sec) n=18	2.42 (1.94-3.36)	1.28-3.79[a]
EAm_t (cm/sec) n=21	5.62 (4.87-7.08)	2.67-7.93
Em_t/Am_t n=18	2.01 (1.54-3.79)	0.73-4.99[a]
$IVRT_t$ (msec) n=39	44 (41-47)	38.9-51.05
Sm_{endo} (cm/sec) n=39	6.01 (4.76-6.94)	3.44-8.70
Em_{endo} (cm/sec) n=18	6.24 (5.22-7.51)	4.06-9.03[a]
Am_{endo} (cm/sec) n=18	3.14 (2.25-3.56)	1.39-4.44[a]
EAm_{endo} (cm/sec) n=21	6.93 (5.88-7.56)	3.45-8.91
Em_{endo}/Am_{endo} N=18	2.20 (1.56-4.07)	0.94-2.66[a]

Variables	**Median (IQR)**	**5th-95th percentiles**
Sm_{epi} (cm/sec) n=39	5.09 (3.91-5.96)	2.50-7.71
Em_{epi} (cm/sec) n=18	4.87 (3.86-5.38)	2.61-6.73[a]
Am_{epi} (cm/sec) n=18	1.99 (1.32-2.78)	0.87-3.27[a]
EAm_{epi} (cm/sec) n=21	5.13 (4.10-6.07)	1.75-6.87
Em_{epi}/Am_{epi} n=18	2.06 (1.32-3.82)	0.91-6.55[a]
Systolic MVG n=39	0.917 (0.676-1.293)	0.321-1.737

6.5 Table 5

Values for systolic colour radial tissue Doppler parameters, separated for French Lops and Dwarf Lop.

Systolic colour radial tissue Doppler values (Sm_t, *P*=0.008; Sm_{endo}, *P*= 0.016; Sm_{epi}, *P*=0.014) were statistically significantly different between both breeds.

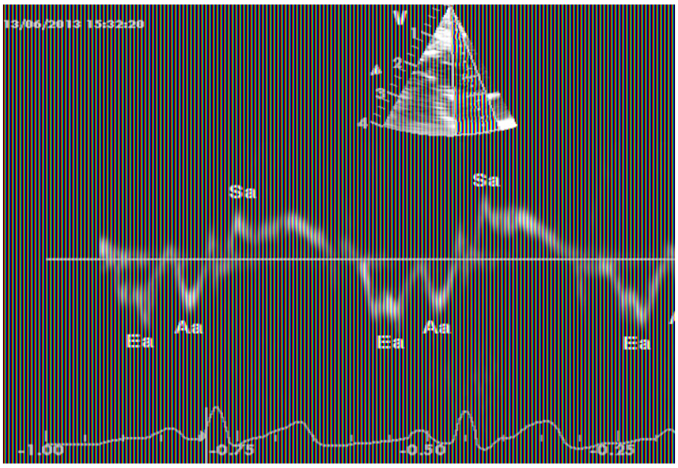
IQR= Interquartile range; cm/sec=centimetres per second; Sm_{endo}=systolic subendocardial wave; Sm_{epi}=systolic subepicardial wave; Sm_t=systolic mid-ventricular wave.

	Dwarf Lop (n=22)		French Lop (n=14)	
Variable	Median (IQR)	Percentiles 10^{th}-90^{th}	Median (IQR)	Percentiles 10^{th}-90th
Sm_t (cm/sec)	4.58 (3.83-5.33)	3.47-7.54	6.18 (5.40-7.11)	4.64-8.0
Sm_{endo} (cm/sec)	5.01 (4.24-6.34)	3.93-7.69	6.49 (5.95-7.00)	5.39-7.88
Sm_{epi} (cm/sec)	4.18 (3.29-5.63)	2.52-6.59	5.75 (4.93-6.28)	3.88-7.13

6.5 Figure 1

Septal mitral annulus longitudinal pulse-wave tissue Doppler trace in one of the rabbits of the study.

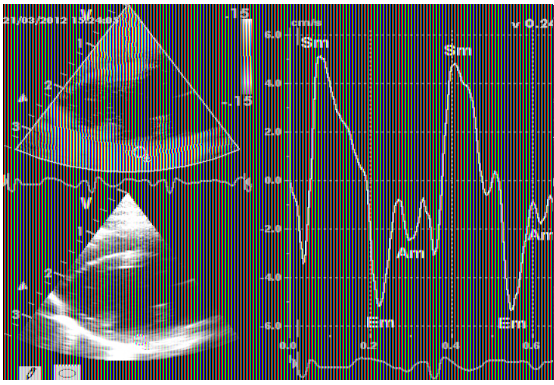
Aa=late diastolic wave; Ea=early diastolic wave; Sa=systolic wave

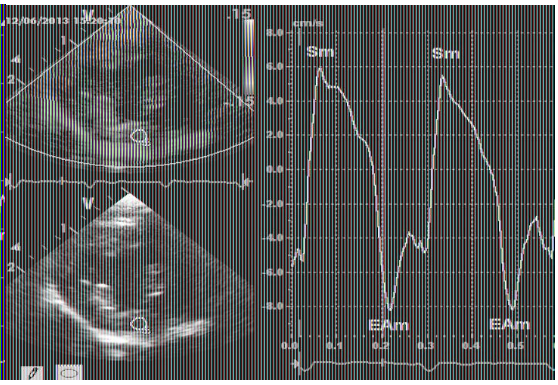


6.5 Figure 2

Radial colour tissue Doppler images from rabbits in the study. Single gate, mid-ventricular with separation (2a) and summation (2b) of early and diastolic wave, respectively; and endocardial and epicardial gates with separation (2c) and summation (2d) of E and A wave, respectively.

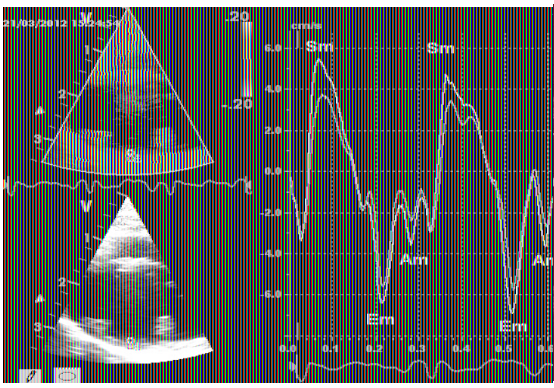
Am=late diastolic wave; Em=early diastolic wave; EAm=summated diastolic waves; Sm=systolic wave

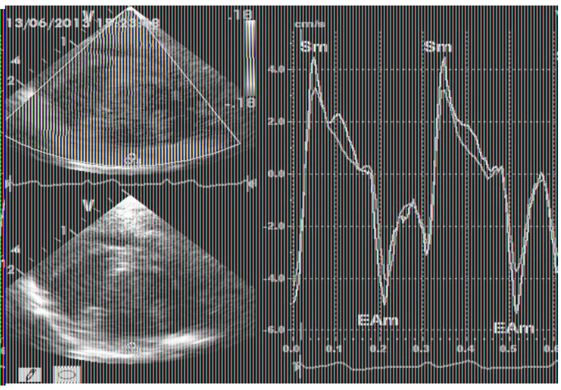




2a

2b





2c

2d