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# Left ventricular radial colour and longitudinal pulsed-wave tissue Doppler echocardiography in 39 healthy domestic pet rabbits

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## ABSTRACT

This paper reports radial colour and longitudinal mitral annulus pulsed-wave tissue Doppler findings in a large cohort of healthy, adult pet rabbits. Thirty-nine rabbits (22 Dwarf Lops, 14 French Lops and three Alaskans) underwent conscious echocardiography. The median age of the rabbits was 22 months and the median weight was 2.8 kg (Dwarf Lop 2.4 kg/French Lop 6.0 kg). Adequate radial colour and longitudinal pulsed-wave tissue Doppler traces were obtained in 100% and 85% of cases, respectively. Most systolic tissue Doppler parameters were significantly higher in French Lops than in Dwarf Lops. Separation of mitral inflow diastolic waves was present in 40% of cases using conventional spectral Doppler and in >60% of cases using pulsed-wave tissue Doppler which could be beneficial when evaluating diastolic function in rabbits. This study can be used as a reference for normal echocardiographic tissue Doppler values for adult rabbits undergoing conscious echocardiography in clinical practice.

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## 1. Introduction

The number of rabbits kept as pet animals in the United Kingdom (UK) is estimated around 1.7 million (Edgar and Mullan, 2011; Mullan and Main, 2006). Almost 60% of these rabbits are registered with a veterinary practice (PDSA, 2011) and general and specialized veterinarians are faced with the potential of a rabbit presenting with cardiac disease (Pariaut, 2009; Reusch, 2006). This results in an increased demand to perform echocardiography in pet rabbits as clinical cases (Pariaut, 2009; Reusch, 2006). Indeed the number of reports describing congenital or acquired cardiac diseases in rabbits (Gava et al., 2013; Marini et al., 1999; Martin et al., 1987; Voros et al., 2011) and anecdotally observed cases from practice (Pariaut, 2009; Reusch, 2006) are growing.

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, 2004b, 2005a, 2005b, 2006a, 2006b; Decloet et al., 2013; Hori et al., 2007; Schwarzwald et al., 2009; Yu et al., 2007). 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 (Boon, 2011; Yu et al., 2007).

To the best of the authors' knowledge, the use of radial colour Doppler 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 (Fontes-Sousa et al., 2009; Nagueh et al., 2000; Stypmann et al., 2007). 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, 2006a) and in the evaluation of conventional echocardiographic measurements when comparing two common rabbit pet breeds, French Lops (FL) and Dwarf Lops (DL) (Casamian-Sorrosal et al., 2014).

The aims of this study were: 1) to evaluate the feasibility of performing conscious echocardiographic TDI examination in a large cohort of healthy pet rabbits; 2) to establish normal values for radial colour TDI and mitral annulus longitudinal pulsed-wave TDI parameters from the same cohort of pet rabbits and 3) to evaluate breed

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and sex differences and a potential correlation of age, weight and heart rate with selected parameters.

## 2. Materials and methods

Ethical approval, which included specific owner consent to a full echocardiography examination, was granted by the University of Bristol Ethical Review Group. Forty-one adult pet rabbits were recruited from pet owners from a local practice or from a local breeder. A full history and physical examination was obtained in all rabbits. Rabbits were excluded from the study if they show a high stress level (subjectively assessed during examination) or any evidence of systemic or cardiac disease on history or physical examination. Breed, age, sex and weight were recorded.

For ECG monitoring during echocardiography, an area of around  $0.5 \times 0.5$  cm was clipped at the dorsal antebrachium bilaterally and either the right or left dorsal metatarsal or dorsal tibial area for the placement of three ECG pads. Additionally, both sides of the lateral thorax were clipped over the area of the apex beat for echocardiography. The rabbits were conscious during the echocardiographic examination, and they were placed in right and left lateral recumbencies on a standard echocardiographic table. A GE Vivid 7 system and a standard phased-array, variable frequency (4.5–11.5 MHz) transducer was used. Images were acquired and analysis was performed at a later stage on the same station by one observer (DCS). Echocardiography was abandoned if a rabbit was considered to be very stressed during echocardiographic examination. This was assessed subjectively by evaluation of their demeanour, including resistance to lateral recumbency, making attempts to rise, or an increase in respiratory depth or rate.

A complete echocardiographic investigation including two dimensional, M-mode, colour Doppler and spectral Doppler flow measurements was performed by one trained observer (DCS). If abnormalities were detected, these rabbits were excluded from the study. Subsequently, radial colour TDI and mitral annulus pulsed-wave TDI was performed by the same observer following standard protocols as described for small animals elsewhere (Boon, 2011; Chetboul et al., 2004a; Simpson et al., 2007). The heart rate (HR) was measured manually (with the specific software tool) for each loop from which measurements were obtained to avoid possible spurious results of the automatic number generated by the machine.

Briefly, Doppler mitral inflow E and A waves were measured as reported previously (Boon, 2011). Summated E and A waves were recorded as EA wave. 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 optimize tissue signal recording. Measurements included lateral and septal peak early diastolic (Ea), late diastolic (Aa) and systolic (Sa) mitral valve annular velocities (Fig. 1). Subsequent calculations of lateral and septal Ea/Aa and E/Ea 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 optimize imaging of the left ventricular endocardium and epicardium. Real-time colour Doppler images were superimposed on the grey scale with a frame rate of  $\geq 200$  frames/s and the gain and velocity range were adjusted to maintain optimal colouring and to avoid aliasing artefacts. Two different recording modes were applied, a single  $2 \times 2$  mm sampling window, which was placed mid-ventricular (t), and two  $1 \times 1$  mm sampling windows, of which one was placed at the endocardium (endo) and one at the epicardium (epi), were used. For each region the following parameters were recorded: systolic velocity ( $Sm_t$ ,  $Sm_{endo}$ ,  $Sm_{epi}$ ), Em wave peak velocity ( $Em_t$ ,  $Em_{endo}$ ,  $Em_{epi}$  respectively), Am wave peak velocity ( $Am_t$ ,  $Am_{endo}$ ,  $Am_{epi}$  respec-

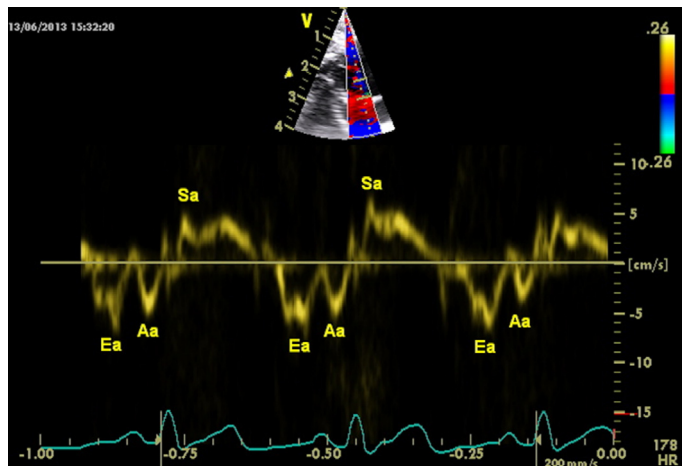


Fig. 1. Septal mitral annulus longitudinal pulsed-wave tissue Doppler trace in one of the rabbits of the study. Aa, late diastolic wave; Ea, early diastolic wave; Sa, systolic wave.

tively), summated Em and Am wave peak velocity ( $EAm_t$ ,  $EAm_{endo}$ ,  $EAm_{epi}$ , respectively) (Fig. 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 these data. Isovolumic relaxation time (IVRT) was measured using the single gate analysis. For all variables, three cardiac cycles were measured and the average was calculated. Adequate quality traces were those with clarity, consistency and good alignment, and any image of inadequate quality was excluded from analysis. Additionally, the average heart rate during examination (HRA) was calculated for each case.

### 2.1. Statistics

A statistical software programme (SPSS 19 for Mac, IBM) was used for analysis. Categorical variables such as breed and sex were described in percentages. A Kolmogorov–Smirnov test was used in all the numerical variables to assess normality with a  $P < 0.05$  taken as a significant deviation from normal. All data, irrespective of their distribution, were reported as median with interquartile range (IQR) and 5th–95th percentiles for homogeneity. In variables with less than 20 values, 10th–90th percentiles were given and this was indicated in the text or table. Differences between breeds (FL vs DL) of the following variables: HRA, weight, age, and echocardiographic variables with more than 30 cases; were compared using t-test for normally distributed data and Mann–Whitney U test for non-normally distributed data. All were not included in the comparison due to low numbers. These same variables (HRA, weight, age and echocardiographic variables with more than 30 cases) were also compared between sexes in the same manner. Variables, which were statistically different between FL and DL were reported separately for both breeds. Correlations between HR and age with echocardiographic variables were calculated in variables with more than 30 cases by Pearson's test if the variables were normally distributed and by Spearman's rho test if one or both were non-normally distributed. Significant correlation was taken at  $P < 0.05$ .

## 3. Results

Of the 41 rabbits examined, two rabbits were excluded, one for an arrhythmia detected on auscultation and another one became stressed and echocardiography was abandoned.

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