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Assessment of right ventricular function in healthy Great Danes and in Great Danes with dilated cardiomyopathy.

--Manuscript Draft--

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Abstract:	Introduction Right ventricular dysfunction is one of the significant negative prognostic determinants in human dilated cardiomyopathy (DCM) patients. Many right ventricular indices are weight dependent. There is paucity of literature on reference values for the right heart in giant breed dogs (over 50kg), including Great Danes (GDs). Objectives To compare indices of right ventricular (RV) function in echocardiographically normal GDs and those with preclinical DCM (PC-DCM) and DCM with congestive heart failure (DCM/CHF). Animals A total of 116 client-owned adult GDs: 74 normal dogs, 31 with PC-DCM and 11 with DCM/CHF. Methods Retrospective, single centre cohort study. RV function was determined using: free wall RV longitudinal strain (RVLS), strain rate, fractional area change (FAC), tricuspid annular plane systolic excursion (TAPSE), and pulsed-wave tissue Doppler imaging-derived systolic myocardial velocity of the lateral tricuspid annulus (TDI S'). Relationships between DCM status and various indices of RV function were explored. Results RV function with TAPSE (P=0.001), FAC (P<0.001) and TDI S' (P<0.001) was decreased in dogs with both PC-DCM and DCM/CHF compared with healthy dogs, with FAC also being lower in DCM/CHF compared with PC-DCM (P=0.048). RVLS was more impaired in the DCM/CHF than PC-DCM group. (P=0.048). On the receiver operating characteristic curve analysis, the area under the curve was highest for RVLS at 0.899 to differentiate between normal vs DCM/CHF. Conclusion As the stage of DCM progressed, echocardiographic variables of RV function including TAPSE, FAC, TDI S', RVSL and strain rate worsened, indicating impaired RV systolic function in GDs affected by DCM.
Response to Reviewers:	

Professor Michele Borgarelli, DMV, PhD, DECVIM-CA (Cardiology)
Editor-in-Chief
Journal of Veterinary Cardiology

Saturday 11th May 2024

Dear Prof. Borgarelli,

We wish to submit the revised version of the manuscript (JVC-D-23-00098R2) entitled "Assessment of right ventricular function in healthy Great Danes and in Great Danes with dilated cardiomyopathy" for consideration by "*Journal of Veterinary Cardiology" (JVC)*.
We are very grateful of the positive feedback we have received, and we wish to thank you and the reviewers for your time, comments, and suggestions. We believe the changes suggested will enhance the manuscript and improve its readability and clarity.

We have made every attempt to address all editor's comments and suggestions in this revised manuscript. We have answered all the editor's comments in the "Response to editor's comments" document.

We hope that our resubmission is now suitable for inclusion in *JVC* and we look forward to hearing from you.

Thank you for your consideration of this manuscript,
Sincerely,

Elzbieta Mederska on behalf of the coauthors
Department of Small Animal Clinical Sciences, Institute of Veterinary Science, University of Liverpool
Email: ela@liverpool.ac.uk

Authors' response to editor's comments

Manuscript Number: JVC-D-23-00098R2

Assessment of right ventricular function in healthy Great Danes and in Great Danes with dilated cardiomyopathy.

The authors thank the editor and reviewers very much for your careful consideration of our manuscript.

I am happy to inform you that I have decided to conditionally accept your manuscript in Journal of Veterinary Cardiology. What this means is that the manuscript has successfully gone through scientific review but there are remaining editorial changes that need to be made before your manuscript will be suitable for publication in the Journal of Veterinary Cardiology. The required changes are listed below.

- Please read carefully the PDF attached to this mail and make proper changes as requested
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Once I receive the revision, I will review it without further consulting external referees. If all editorial comments have been successfully addressed, the manuscript will be officially accepted and forwarded to the production department for typesetting.

I also ask that you carefully proofread the entire manuscript to eliminate all typographical errors and imprecision before submitting your revised manuscript. Please consult the Guide to Authors on the Journal's web site, making sure your final submission conforms to all the Journal's style guidelines

I look forward to receiving your revised manuscript as soon as possible. Please submit your revised manuscript within 10 days or your manuscript will withdraw from the system.

Kind regards,

Michele Borgarelli
Editor-in-Chief
Journal of Veterinary Cardiology

Thank you very much for your careful consideration of our manuscript. We have now responded to the editor's comments as below, and have revised our manuscript according to your constructive feedback.

- Please read carefully the PDF attached to this mail and make proper changes as requested

Response: We appreciate your feedback. We have addressed the suggested changes throughout the manuscript, with the exception of the note on line 13 of the Title page regarding the relocation of the sentence ‘Some of the results were presented as an abstract at the Veterinary Cardiovascular Society November meeting, UK, November 2022’. As per The JVC Guide to authors, specifically the title page section, it is recommended to indicate the date and location of data presentation, if relevant, at the bottom of the page, without the use of a footnote.

- You have labelled table 1-4 twice. It looks like you have 8 tables in your manuscript, please labels them accordingly

Response: Thank you for your comment. We acknowledge that there are indeed only four tables included in the manuscript. However, they appear duplicated in the submission, as they are both attached at the end of the manuscript as per the JVC Guide to Authors, and also provided separately as individual tables.

- when resubmitting your manuscript be sure to upload a clean version of it (no track changes or highlights)

Response: The clean version has been now submitted.

Title: Assessment of right ventricular function in healthy Great Danes and in Great Danes with dilated cardiomyopathy.

Short Title: Right ventricular function in dilated cardiomyopathy

Elzbieta Mederska DVM[a], Hannah Stephenson BVMS[a,b] , Thomas W Maddox PhD[a], Joanna Dukes-McEwan PhD[a]

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Acknowledgements:

This study was supported by grants from the European Commission (LUPA-GA 201270; the LUPA project), the Great Dane Breed Council (UK), and the Kennel Club Charitable Trust (UK). In addition, it was supported by donations from Great Dane owners and private benefactors. The work would not have been possible without the support and participation of Great Dane owners and their dogs. The authors are especially grateful to Joan Toohey for her administration and coordination of the project. Members of the cardiology service, past and present, all participated in the work with Great Danes.

Some of the results were presented as an abstract at the Veterinary Cardiovascular Society November meeting, UK, November 2022.

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ABSTRACT

Introduction

Right ventricular (RV) dysfunction is a significant negative prognostic indicator in human dilated cardiomyopathy (DCM). Many right ventricular indices are weight dependent. There is paucity of literature on reference values for the right heart in giant breed dogs (over 50kg), including Great Danes (GDs).

Objectives

To compare indices of RV function in echocardiographically normal GDs and those with preclinical DCM (PC-DCM) and DCM with congestive heart failure (DCM/CHF).

Animals

A total of 116 client-owned adult GDs: 74 normal dogs, 31 with PC-DCM and 11 with DCM/CHF.

Methods

Retrospective, single centre cohort study. Right ventricular function was determined using: free wall RV longitudinal strain (RVLS), strain rate, fractional area change (FAC), tricuspid annular plane systolic excursion (TAPSE), and pulsed-wave tissue Doppler imaging-derived systolic myocardial velocity of the lateral tricuspid annulus (TDI S’). Relationships between DCM status and indices of RV function were explored.

Results

Right ventricular function with TAPSE ($P=0.001$), FAC ($P<0.001$) and TDI S’ ($P<0.001$) was decreased in dogs with both PC-DCM and DCM/CHF compared with

healthy dogs, with FAC also being lower in DCM/CHF compared with PC-DCM (P=0.048). The RVLS was more impaired in the DCM/CHF than PC-DCM group. (P=0.048). On the receiver operating characteristic curve analysis, the area under the curve was highest for RVLS at 0.899 to differentiate between normal vs DCM/CHF.

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Short Title: Right ventricular function in dilated cardiomyopathy

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KEY WORDS: Speckle tracking echocardiography, Tissue Doppler imaging, myocardial strain, Tricuspid Annular Plane Systolic Excursion (TAPSE), Fractional Area Change (FAC)

TABLE OF ABBREVIATIONS

AUC	area under the curve
cMRI	cardiac magnetic resonance imaging
DCM	dilated cardiomyopathy
DCM-CHF	dilated cardiomyopathy with congestive heart failure (clinical DCM)
FAC	right ventricular fractional area change
GDs	Great Danes
PC-DCM	pre-clinical dilated cardiomyopathy
R-CHF	right sided congestive heart failure
ROC	receiver operating characteristic curve
RV	right ventricle
RVLS	right ventricular longitudinal systolic strain of the right ventricular free wall

TAPSE	tricuspid annular plane systolic excursion
TDI S’	pulsed wave Tissue Doppler Imaging derived peak velocity of the systolic wave of the lateral tricuspid annulus

INTRODUCTION

Dilated cardiomyopathy (DCM) is the second most common canine acquired cardiac disease, and the most common in large breed dogs [1]. It is a primary heart muscle disease characterized by left or biventricular systolic impairment [2]. Historically, most clinical attention has been devoted to evaluating left ventricular function and morphology. In contrast, the right ventricle (RV) has only recently been considered and evaluated in human DCM or heart failure patients [3–7]. This can be partly explained by challenges imposed by complex RV anatomy and function, limiting its evaluation by standard echocardiography [8]. The impact of RV function on survival in patients with severe left heart failure has been emphasized by studies showing that LV function loses its prognostic value in patients with an left ventricular ejection fraction less than 25%, whereas a preserved right ventricular ejection fraction (≥35%) estimated by radionuclide ventriculography proved to be predictive for exercise capacity and survival, even in advanced heart failure [5]. Baseline right ventricular ejection fraction <20% estimated by gated-equilibrium radionuclide ventriculography is a significant independent predictor of mortality and heart failure resulting in hospitalization in systolic heart failure [6]. The combination of RV systolic and diastolic functional parameters represents a very powerful tool for risk stratification of patients with symptomatic heart failure due to idiopathic DCM [7,9]. Chetboul et al. (2007) described that, alongside left ventricular systolic dysfunction, RV systolic function is also impaired in dogs with DCM and that one-third of the dogs with right systolic Tissue Doppler Imaging alterations did not show any right heart dilatation on conventional echocardiography [10]. Right ventricular morphology cannot accurately predict RV function in dogs [11] . Several investigators have used allometric scaling to normalize indices for body size, but only for dogs up to a maximum of 64.5kg [10–14]. Many right ventricular indices are weight dependent in dogs and data are lacking for giant breed dogs. There is also a need for breed specific reference ranges for the right heart in Great Dane dogs (GDs), similar to those available for the left heart [15].

The purpose of this study was to compare indices of RV function in echocardiographically normal GDs and those with pre-clinical (PC-DCM) and clinical dilated cardiomyopathy (DCM-CHF). It was hypothesised that the RV systolic function was lower in GDs with DCM than in healthy dogs and the RV indices would be associated with body weight.

ANIMALS, MATERIALS AND METHODS:

This prospective, observational study was performed at the Small Animal Teaching Hospital, University of Liverpool, which has been active since January 2008. The right heart data were retrospectively retrieved up to January 2023 by one of the authors (EM). In assessment of RV function, amendments of the existing ethical approval of ongoing study of GDs (VREC464) was not required by the University of Liverpool Committee on Research Ethics since owners were not being contacted and no additional samples were taken.

The dogs included in the study were client- or breeder-owned and were examined as part of a screening program for DCM. The apparently healthy dogs screened had to be over four years of age. Clinical cases could be younger, but over 12 months old. All dogs underwent a full physical examination and had haematology and biochemistry analyses. Thyroid function was initially tested only in dogs with clinical suspicion of hypothyroidism and later in all dogs.

Dogs were excluded if significant systemic disease was identified from the physical examination and blood work. Dogs with conditions resulting in pressure or volume overload other than DCM were excluded. Trivial mitral regurgitation alone or associated with myxomatous degenerative valvular changes was permitted provided there was no left atrial or left ventricular enlargement. Dogs with physiologic tricuspid or pulmonary valve regurgitation were not excluded due to the high prevalence of this finding in healthy dogs. Dogs were divided into three categories: normal, PC-DCM and DCM-CHF as described previously based on echocardiography [15,16].

Dogs were not included if they had an uncooperative temperament that required sedation for any study procedure. Dogs with clinically important brady- or tachyarrhythmias, defined as those requiring antiarrhythmic therapy by the attending cardiologist, were excluded from the normal group, but could be included in the DCM groups if the arrhythmia was considered a manifestation of DCM. Systemic hypertension defined as a systolic arterial blood pressure >180 mm Hg by Doppler sphygmomanometry was also an exclusion criterion.

Great Danes with equivocal echocardiographic results, not fully meeting criteria to diagnose DCM or healthy status, were also excluded from analysis. Dogs with atrial fibrillation were not permitted in the healthy group but could be included in pre-clinical and clinical DCM group with confirmatory echocardiographic findings. Antiarrhythmics, pimobendan, ACE inhibitors and furosemide were permitted in the PC-DCM and DCM-CHF groups, but not in the normal group. Hypothyroid dogs in all groups had to be stable on thyroid supplementation for > two months to be included. Signalment data were retrieved including sex, neuter status, age at the time of the assessment, and body weight. Echocardiographic[c] examinations were conducted in unsedated GDs lying in right and left lateral recumbency on an echocardiographic table with simultaneous ECG monitoring as previously described [15]. At least three cardiac cycles were acquired from each imaging plane. Examinations were acquired by three study investigators, which included a board-certified cardiologist (JDM) and two cardiology residents (EM, HS) working under supervision of a Diplomate. All echocardiographic studies were measured at the time of assessment by the echocardiographer [15]. The right heart studies were measured offline[d] retrospectively by the first author (EM).

A left apical four-chamber view optimized for the RV was used for the acquisition of images from which the following indices of RV systolic function were obtained: tricuspid annular plane systolic excursion (TAPSE), RV fractional area change (FAC), pulsed wave Tissue Doppler Imaging derived systolic myocardial velocity of the lateral tricuspid annulus (TDI S'), and speckle-tracking echocardiography-derived global longitudinal RV free wall systolic strain (RVLS) and strain rate (RV strain rate). Those indices have been shown to have low intra observer variability in previous studies [11,14,17,18].

Values for each echocardiographic parameter consisted of the mean of three to five consecutive measurements. In this retrospective review of images acquired, only adequate right heart images were accepted. The number of studies in which each right heart variable could be assessed was documented. Views with left ventricular outflow tract were avoided in order to reduce foreshortening of the RV and to maximize the RV longitudinal dimension. Images were selected with the RV lumen appearing triangular, avoiding a crescent shaped RV. Tracing of the RV endocardium at end-systole and end-diastole were performed by either identifying the opening and closing of the tricuspid valve or by visually assessing the smallest and largest RV chamber size. The anatomic M-mode technique was used frequently on stored 2D cine loops in the early years of the study as TAPSE was not initially part of the standard echocardiography protocol at the time [19].

Strain and strain rate measurements were calculated with 2D speckle-tracking software[c] using the left ventricular algorithm, as no defined RV speckle-tracking echocardiography algorithm was available to the authors. Only RV free wall longitudinal strain and strain rate were analyzed as longitudinal motion as recommended by human guidelines [20]. The right ventricular free-wall analysis was performed by tracing the endocardial border of the myocardium from the lateral tricuspid annulus to the RV apex to select the appropriate region of interest (ROI), which was automatically detected by the software. When necessary, manual adjustments were performed to include and track the entire RV free wall myocardium over the cardiac cycle. When the automated software could not track the myocardial regions, the regions of interest were retraced and recalculated. The right ventricular longitudinal systolic strain was defined as the absolute peak negative value of the global strain wave. The right ventricular strain rate was defined as the absolute value of the negative peak value of the strain rate wave [21]. For 2D strain measurement imaging, images were selected which were at least 50 frames per second (fps).

The age, sex, neuter status and body weight were obtained from the medical records. Clinical status at the time of diagnosis, echocardiographic findings and measurements, and any outcome data included in the record were also recorded. For dogs with multiple serial assessments in the healthy group, only the last visit was used for statistical analysis. For dogs with pre-clinical and clinical DCM the first visit at the time of diagnosis was used.

Statistical analysis

The normality of data distribution was tested using the Shapiro-Wilk test and graphically. Continuous variables were reported as mean ± standard deviation for normally distributed data and median (25th - 75th percentile) for non-normally distributed data. Differences between DCM status and various indices of RV function were explored by one way ANOVA (normally distributed data) or Kruskal Wallis test (non-parametric) with post-hoc pairwise testing by Bonferroni Post-hoc-Tests and Dunn's methods, respectively. Between-group analysis (non- paired groups) was performed using two-sample t- test for continuous parametric data, Mann Whitney U-test for non-parametric data and chi-squared test for categorical variables. Pearson correlation coefficients were calculated for normally distributed echocardiographic variables and Spearman correlation coefficients for non-normally disturbed variables to explore the relationship between the indices of RV systolic function and bodyweight (in kg), age (in years), and heart rate (as R-R interval, in ms). Outliers, defined as points further away than one and half times the interquartile range (values above and below the whiskers in boxplots), were included in all analyses; missing data were omitted. For any right heart variables that were different between the normal group and the DCM (both all DCM and clinical DCM considered separately) groups, receiver operating characteristic (ROC) curves were constructed and area under the curves (AUC) reported. Various cut-offs to optimize sensitivity, specificity or the optimal combination of both were explored.

Statistical analyses were performed using commercially available software[e,f]. Reference intervals for the echocardiographic data derived from normal dogs were calculated within the R statistical environment[g] using the *referenceIntervals* package with the robust method applied, with 90% confidence intervals for these generated through a bootstrapping procedure. A P value of <0.05 was considered significant.

RESULTS

From the database, between January 2008 and January 2023, a total of 229 GDs were identified. Eighty-six dogs were excluded, due to equivocal classification based on echocardiography. A further 27 dogs were excluded (Figure 1). In total 116 dogs fulfilled inclusion criteria with 59 male (42 entire) and 57 females (40 entire). There were 74 dogs in the normal category, 31 in PC-DCM and 11 in DCM-CHF groups. Their details are included in Table 1. The median age was 6.08 years (interquartile range: 4.6-7.5 years) and the normal dogs were younger than PC-DCM and DCM dogs. Median weight was 64.65 kg (interquartile range: 58.7-70.9kg), which was not different between groups, but there was a difference in weight between males (median weight 69kg, interquartile range: 63.75- 74.8) and females (median weight 60.2kg, interquartile range: 55.4-64.8) ($P<0.001$). There was a difference in sex between the groups with predominance of males with PC-DCM and DCM-CHF, and slightly more females in the healthy group (Table 1).

In the normal group, a number of dogs received medication. Five dogs received non-steroidal anti-inflammatory medication and one levothyroxine sodium. Seven dogs in the PC-DCM group were receiving medication (3/7 benazepril, 2/7 digoxin, 2/7 pimobendan, 1/7 meloxicam, 1/7 metronidazole and 1/7 levothyroxine sodium). In the DCM-CHF group, all dogs had left sided congestive heart failure, either compensated on heart failure medication or decompensated at the time of the initial assessment. Three DCM-CHF dogs had clinical signs of right sided congestive heart failure (3/3 pleural effusion and one also had ascites) as well as pulmonary oedema. At the time of presentation in the DCM-CHF group, six dogs received pimobendan and additionally 1/11 dog mexiletine and sotalol, 4/11 furosemide, 1/11 benazepril and digoxin and 1/11 diltiazem. Atrial fibrillation was diagnosed in 5/11 dogs in the

DCM-CHF group and 4/31 dogs with PC-DCM and was an exclusion for the normal group.

In our study of 74 normal GDs, we measured five RV echocardiographic variables. Reference values for five echocardiographic indices of RV systolic function assessed in 74 conscious healthy GDs are provided in Table 2.

When the normal group was considered, there were no differences between sexes for TAPSE (P=0.445), TDI S' (P=0.19), FAC (P=0.06), RVLS (P=0.825) or RV strain rate (P= 0.204). There was no interaction between neuter status and sex (P=0.746). There was no association between right heart echo variables and weight or age (Table 3). Therefore, the right heart variables were not normalized for body weight in this study. In the normal group, exploration of associations between RV function variables and R-R interval did not show any significant influence of heart rate on these variables, except RV strain rate (Table 3).

The right heart variables measured or calculated in the three groups are shown in Table 1. There was evidence of decreased RV systolic function in both DCM groups (Figure 2).

Receiver operating characteristic curve analyses for selected variables are presented in Table 4. All variables showed good discriminatory ability to differentiate between the normal group vs. DCM-CHF and normal vs PC-DCM+DCM-CHF. All variables exhibited an AUC exceeding 0.5, with actual values ranging from 0.718 to 0.889 (P<0.05 for all). However, the highest AUC was for RVLS at 0.899 (confidence intervals: 0.783-0.995) to differentiate between normal vs DCM-CHF (Figure 3).

There was no difference for TAPSE (P=0.762), TDI S' (P=0.537), FAC % (P=0.498), RV strain (P= 0.76) and RV strain rate (P=0.262) between dogs receiving pimobendan and those that did not in both DCM groups. There was also no difference between dogs in atrial fibrillation and those in sinus rhythm regarding RV systolic function echocardiographic variables.

DISCUSSION

In this study, we described indices of RV function in healthy GDs and those affected by DCM. From the 74 echocardiographically normal GDs, we established reference values and used them for comparative analysis. To the authors' knowledge, these are the first right heart echocardiographic variables described for a giant breed in health and with DCM. For the right heart variables, there was no association with body weight, age or R-R interval.

In previous studies, only single dogs representing large or giant breeds have been used to generate reference intervals with maximal weight of 66kg in the study by Feldhütter et al, and 45kg in that of Visser et al [12,14]. Therefore, the utility of normalisation of these values in larger breed dogs was unknown. In the context of GDs, a breed known for its remarkable variation in size (our group ranged from 48.5 to 93kg), it is noteworthy that despite this diversity there were no associations between body weight and the right heart variables. Therefore, the breed-specific reference intervals provided for the normal GDs in this study are of more benefit than weight-normalised values.

Although DCM is predominantly considered to be a left heart disease, and the two DCM groups needed to meet the criteria for diagnosis of DCM with left ventricular dilatation and impaired LV systolic function, we provide evidence in this study that RV function is also impaired in GDs. The right ventricle may be affected by various cardiovascular diseases, including those traditionally viewed as left heart specific.

Recently, there has been more awareness of the importance of biventricular assessment in DCM in humans [4]. Most recent research showed that RV involvement is far from uncommon in DCM. Several mechanisms such as the primary cardiomyopathic process, ischaemia, pulmonary hypertension, decreased RV coronary perfusion by a failing LV and LV dilation restricting RV diastolic function have been proposed [4]. Right ventricular systolic dysfunction independently serves as a robust predictor of transplant-free survival and unfavorable outcomes in individuals with DCM [22]. Additional research involving longitudinal studies in Great Danes is imperative to ascertain the prognostic value of right heart variables.

All the variables showed that RV function deteriorated (and strain and strain rate were less negative) as the stage of the disease progressed. From the ROC analysis, there was fair to good accuracy in differentiating between normal and DCM, superior for the clinical DCM group, with all AUCs over 0.7. The individual variable with the greatest discriminatory ability in Great Danes in this study was RVLS between normal and DCM-CHF group.

Unlike the left ventricle, the RV myocardium exhibits a simpler structure with only two discernible layers. The thinner outer layer primarily consists of circumferentially oriented fibers, accounting for approximately 25% of its composition. In contrast, the inner layer, which is more prominent, is predominantly comprised of longitudinally oriented fibers [8]. Tricuspid annular plane systolic excursion represents a measure of RV longitudinal function and can provide an objective estimate of RV systolic function [23]. In human patients with congestive heart failure, TAPSE measuring 14 mm or less was found to be significantly linked to an elevated risk of either all-cause death or heart transplantation, adding significant prognostic information to the NYHA clinical classification, the echocardiographic evaluation of left ventricular function and to mitral Doppler variables [3]. Additionally, TAPSE <15.1 mm has been proven to be independent predictor of time to cardiac death in Boxers with arrhythmogenic right ventricular cardiomyopathy [24]. It is also decreased in dogs with severe pulmonary hypertension [25–27].

Furthermore, the measurement of TAPSE is easy to obtain in all patients, irrespective of heart rate and rhythm [3]. Tricuspid Annular Plane Systolic Excursion was reduced in dogs with DCM compared to controls but was not able to differentiate between pre-clinical and clinical DCM. It also had lower AUC than the other RV variables from our ROC analyses. Tricuspid Annular Plane Systolic Excursion should therefore not be used as the sole indicator of RV function or prognosis in DCM in GDs. Since TAPSE was reduced in the PC-DCM group, this implies that RV function may become impaired in the early stages of the disease, well before the onset of CHF. The lack of differences in TAPSE between pre-clinical and clinical DCM groups highlights the need for additional markers to identify RV dysfunction in the early phase and clinical phase of DCM in GDs.

In our study, despite a downward trend of TDI S’ between normal, PC-DCM and DCM-CHF groups, the only pairwise differences were between the normal and both DCM groups, so TDI S’ also did not discriminate between the DCM groups based on the cases included. However, this may be a due to a type II statistical error because of small group sizes for the DCM groups.

In our study, FAC declined with progression of the disease and was statistically different between all groups. Although this was not a longitudinal study, it is possible that FAC has prognostic value or may identify risk of developing right sided congestive heart failure (R-CHF).

Advantages of FAC assessment for right heart function include its relative acquisition angle independence, and that it incorporates an additional plane for functional assessment (i.e. it assesses both the radial and longitudinal planes). Fractional area change correlates with RV ejection fraction by cardiac magnetic resonance imaging (cMRI) most likely due to assessment of global RV systolic function rather than merely longitudinal function assessed by TAPSE and TDI S’ [28]. Fractional area change demands high-quality imaging for accurate endomyocardial border detection, which represents a major shortcoming for this index in some patients, including our study, where it was not possible to obtain it in all patients. During our research, it became evident that obtaining comprehensive echocardiographic variables was impeded by suboptimal imaging, especially in the studies from earlier years where RV optimization and assessment were not part of standard echocardiographic breed screening. In a recent study, FAC and TDI S’ appear to be the most reliable predictors of RV systolic function when compared to cMRI under anaesthesia in dogs [17]. According to the recent study by Kawata et al., human patients in advanced heart failure with DCM with FAC <26.7% had a significantly worse one-year outcome [29]. It is possible that FAC offers more valuable prognostic insights than either TAPSE or TDI S' measurements.

Speckle tracking derived strain and strain rate are useful variables for estimating RV global and regional systolic function. Longitudinal strain is calculated as the percentage of systolic shortening of the RV free wall from base to apex, while longitudinal strain rate is the rate of this shortening. RV longitudinal strain is less confounded by overall heart motion but depends on RV loading conditions as well as RV size and shape. In addition to image resolution (needs as high a frame rate as possible), overall image quality greatly influences the quality of tracking [21].

The main advantage of strain measurement using speckle tracking echocardiography over conventional indices such as TAPSE or TDI S' is angle independence and the possibility of evaluating the entire RV in more than one dimension and not only regional segments. This feature may enable changes to be detected at an earlier stage. Additionally, in humans, RVLS has been shown to have prognostic value in DCM patients [30]. This recent meta-analysis showed that with each 1% worsening in RV global longitudinal strain and RVLS was independently associated with increased risk of all-cause mortality and the composite outcome in patient with heart failure. In our study RVLS tended to decrease (i.e. become less negative) across groups, a change which was significant overall. However, the pairwise testing did not identify differences between the normal and preclinical DCM-CHF group, although there were between normal and DCM-CHF groups. It is possible that this finding shows RVLS is impaired later in the course of the disease. The free wall RV longitudinal strain had the highest AUC for discriminating between normal and clinical DCM at 0.889 and both DCM groups (0.781; data not shown) from our ROC analysis compared with the other right heart variables.

In our study, there were no differences in the right heart variables between dogs receiving pimobendan from those who did not, in the DCM-CHF group. As a positive inotrope, use of pimobendan might be expected to affect indices of RV function. Previous studies have reported contradictory findings with regards to pimobendan treatment, with no significant differences observed in one recent study between dogs with pulmonary hypertension that received pimobendan and those that did not for any RV variable [27]. This is in contrast to the study by Visser et al. (2015), which showed RV ventricular systolic indices had significant increase from baseline following one dose of pimobendan; however, those were healthy dogs [13].

Experimental studies have shown that inotropic stimulation has a more pronounced effect on the infundibular (outflow) region, which is not captured by any of the variables we assessed [31].

Only 3/11 clinical DCM dogs in this study showed clinical signs of R-CHF. With so few dogs with R-CHF, determining if they exhibit further decline in right heart size or function compared to asymptomatic dogs or those with isolated left sided congestive heart failure remains inconclusive. Additional studies with a larger number of animals are needed for this purpose. It is noteworthy that two of the GDs with R-CHF were in fast atrial fibrillation with heart rate of about 180 bpm in the clinics. It is postulated that the sustained elevation in heart rate may elicit deleterious effects on the myocardial structure and function over time, including the right heart, and contribute to development of right sided congestive heart failure [32,33]. These three cases did not undergo 24-hour ambulatory monitoring. Heart rate is an important factor influencing cardiac loading conditions. Increased HR would reduce the preload (i.e. venous return) to the right heart, which could underestimate the RV function. Indeed, this was confirmed in an experimental study with healthy dogs with pacing up to 180 bpm which showed decrease in the RV FAC, TAPSE, TDI S', and RV strain, and might underestimate the RV function [34]. Despite a wide range of heart rates in our study there was no statistical association between R-R interval and the four right heart echocardiographic variables in the healthy GDs, except strain rate. Therefore, correction for a physiologically appropriate heart rate is not required. When it comes to the relationship between strain rate and heart rate, there is not a direct correlation where strain rate consistently increases or decreases with heart rate [35]. In this study, in the healthy group, there was decrease in the RV strain rate with faster HR which is in contrast to the previous study also in healthy dogs [14].

407 It should be noted that the assessed echocardiographic indices of RV function in this study share similar limitations as in other studies, regarding their load dependence and uniplanar assessment (e.g. most only assess longitudinal function and exclude assessment of the RV infundibular region), and many lack validation studies in dogs comparing indices with gold standards such as cMRI. In humans, often no single parameter is sufficient, and a holistic approach is required to assess RV function [36]. The authors would recommend a complete right heart assessment using the available echocardiographic tools as presented here.

415 This was not a longitudinal study; each dog was included only once. Therefore, this study cannot comment on whether RV systolic dysfunction is a predictor of R-CHF in DCM in GDs. In addition, it is imperative to conduct further investigations in canine patients afflicted with DCM to ascertain the significance of each variable in terms of prognosis. It is important to note that this study was not specifically designed to examine prognostic outcomes but rather aimed to explore the variables independently.

422 This study has a number of limitations. One notable limitation is the small sample size within the DCM group, which restricts the statistical power and generalizability of the findings. The retrospective nature of the study as far as the right heart assessment was concerned led to missing data points in some dogs, as early echocardiographic examinations were not optimized to specifically evaluate indices of right sided function or RV size. It is worth noting that the use of low-frequency matrix M4S or M5S transducers[c] in our study often resulted in lower frame rates, typically around 50 frames per second (fps), even with sector angle narrowed as much as possible. Therefore, several variables could not be assessed in all patients due to inadequate echocardiographic acquisition or image quality. Cardiac magnetic

resonance imaging has been regarded as the gold standard in assessment of human RV [37]. Recently, advanced imaging techniques such as cMRI and 3D echocardiography have been described in the evaluation of dogs [12,17]. However, these methods are time-consuming, not readily available, and the equipment is expensive [38]. Dogs also require general anesthesia for cMRI, which will affect function and might pose a risk in clinical cases. Therefore, we have focused on 2D measurements of the right heart in this study. Future work with 3D echocardiography or even cMRI may provide more information about the role of the RV in Great Dane DCM.

Finally, despite this study being a part of a longitudinal study, with most dogs and their outcome being recorded, it is possible that some normal dogs may go on to develop DCM following manuscript preparation. The healthy dogs included were not young (median age 5.63 years) and the most recent examination confirming health was used in this study, to try and prevent inadvertent inclusion of GDs likely to develop DCM, and all equivocal dogs were excluded, which made this less likely. It was not a study aim to explore any relationship between RV function and survival in the GD population, but this could be the subject for future work.

CONCLUSION

We have described the RV systolic function for apparently healthy GDs, and those with DCM. As the stage of the disease progressed, changes in echo variables estimating RV function like TAPSE, FAC, TDI S' and RVSL suggested worsening RV systolic function in GDs affected by DCM. These factors might have consequences concerning treatment and prognosis in dogs with DCM, which should be evaluated in future studies.

Footnotes:

[c] Vivid 7 and E95, General Electric Medical Systems (GE), Buckinghamshire, UK equipped with a 2-4 MHz multifrequency matrix transducers

[d] Echopac; GE, Buckinghamshire, UK

[e] DATAtab: Online Statistics Calculator. DATAtab e.U. Graz, Austria. URL https://datatab.net

[f] SPSS: IBM SPSS Statistics for Windows, Version 29.0. Armonk, NY).

[g] R3.4.1, R Core Team (2023) R Foundation for Statistical Computing, Vienna, Austria

Conflict of interest statement

None of the authors have any conflicts of interest of relevance for this manuscript, which are not declared in the acknowledgements.

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TABLE 1

Comparison of clinical and echocardiographic variables of all dogs. Values reported as median with 25th - 75th percentile.

	Normal	**N**	**Pre-clinical DCM**	**N**	**DCM-CHF**	**N**	**P value**
Number of dogs	74		31		11		
Sex (Male/Female)	29/45		20/11		10/1		**0.001**
Body Weight (kg)	63.1 (57.93-68.68)		64.8 (53.3-91.1)		69 (55.5-95.6)		0.92
Age (years)	5.63 (4.25- 7.17)		6.67 (4-11.75)[a]		6.42 (2.83-10.08)[a]		**0.042**
TAPSE (mm)	20.05 (18.6-22.98)	51	16.6 (15.5-21.52) [a]	24	15.45 (11.08-18.83) [a]	10	**0.001**
TDI S' (cm/s)	18.4 (14.93 – 21.45)	74	14.4 (12-17.7) [a]	31	12.3 (8.7-15.75) [a]	11	**<0.001**
FAC (%)	45.8 (41.1-51.9)	41	40.05 (37.26-47.58)	20	33.05 (25.53-49.58) [ab]	10	**<0.001**
RVLS x (-1%)	23.33 (27.95-21.50)	26	21.71 (23.66-17.43)	7	12.19 (15.16-9.39) [ab]	9	**0.001**

a – p<0.05 compared to normal group

b – p<0.05 compared to pre-clinical group

Abbreviations:

DCM: dilated cardiomyopathy; DCM-CHF: dilated cardiomyopathy with congestive heart failure; FAC: right ventricular fractional area change; N: number of animals; PC-DCM: pre-clinical dilated cardiomyopathy; RV: right ventricle; RVLS: right ventricular longitudinal strain of the right ventricular free wall; TAPSE: tricuspid annular plane systolic excursion; TDI S': pulsed wave tissue Doppler imaging derived peak velocity of the systolic wave of the lateral tricuspid annulus.

TABLE 2

Reference values for five echocardiographic indices of right ventricular systolic function assessed in 74 conscious healthy Great Danes (GDs).

RV function index	**Lower Reference interval**	***Lower 90%CI***	***Upper 90%CI***	**Upper Reference interval**	***Lower 90%CI***	***Upper 90%CI***
TAPSE mm	12.55	*11.04*	*14.23*	27.58	*25.63*	*29.60*
TDI S' cm/s	8.83	*7.49*	*10.10*	28.32	*26.53*	*30.31*
FAC %	30.92	*27.96*	*33.89*	60.57	*57.38*	*64.12*
RVLS x -1%	12.15	*9.04*	*14.66*	36.23	*33.21*	*40.26*
RVStrain rate x -1/s	0.524	*-0.11*	*0.96*	5.58	*4.97*	*6.58*

Abbreviations: CI: confidence intervals; FAC: right ventricular fractional area change; RV: right ventricle; RVLS: right ventricular longitudinal strain of the right ventricular free wall; TASPE: tricuspid annular plane systolic excursion; TDI S': pulsed wave tissue Doppler imaging derived peak velocity of the systolic wave of the lateral tricuspid annulus.

TABLE 3

Associations between right heart echocardiographic variables in 74 conscious healthy Great Danes with age, weight, and RR interval.

	age in years		**weight in kg**		**RR in ms**	
	r	P	r	P	r	P
TAPSE mm	0.01	0.938	0.1	0.471	-0.01	0.955
TDI S' cm/s	-0.06	0.603	-0.02	0.874	-0.18	0.148
FAC %	0.17	0.295	-0.14	0.392	-0.19	0.267
RVLS x-1%	-0.02	0.938	0.07	0.737	0.34	0.119
RV strain rate x-1/s	0.22	0.281	-0.06	0.786	0.54	**0.01**

Abbreviations:

FAC: RV fractional area change; $p < 0.05$, statistically significant; r: correlation coefficient; RV: right ventricle; RVLS: right ventricular longitudinal strain of the right ventricular free wall; TASPE: tricuspid annular plane systolic excursion; TDI S : pulsed wave tissue Doppler imaging derived peak velocity of the systolic wave of the lateral tricuspid annulus.

Table 4

Right ventricle echocardiographic variables: Receiver operator characteristic curve analysis to discriminate between echocardiographically normal Great Danes compared to those with dilated cardiomyopathy with congestive heart failure: selected cut-offs.

	AUC	95% CI AUC	Cut-off	SE%	SP%
TAPSE mm	0.737	0.543-0.932	<19.6	90	49.3
			<15.0	50	90.7
			<14.4	50	94.7
TDI S' cm/s	0.759	0.595-0.924	<18.3	90.9	41.9
			<11.8	45.5	91.4
			<13.8	63.6	81.0
FAC %	0.82	0.651-0.954	<44.6	90	47.5
			<35.9	60	90.2
			<34	60	93.4
RVSL x -1%	0.889	0.783-0.995	>22.4	100	60.6
			>13.3	55.6	90.9
			>16.3	88.9	87.9

In the table, for each variable, three cut-offs were selected which gave sensitivity (≥90%) (1^{st}), specificity (≥90%) (2^{nd}) and the cut-off with the highest Youden index (3^{rd}) in detecting clinical DCM dogs.

Abbreviations:

AUC: area under the curve; CI: confidence interval; FAC: right ventricular fractional area change; RVLS: right ventricular longitudinal strain of the right ventricular free wall; SE: sensitivity; SP: specificity; TAPSE: tricuspid annular plane systolic excursion; TDI S': pulsed wave tissue Doppler imaging derived peak velocity of the systolic wave of the lateral tricuspid annulus.

Figure legends

Figure 1. Selection process.

Abbreviations:

DCM: dilated cardiomyopathy; DCM-CHF: dilated cardiomyopathy with congestive heart failure; n: number of dogs; PC-DCM: pre-clinical dilated cardiomyopathy

Figure 2. Box-and-whisker plots for five right ventricular function indices comparison between three groups. Each box represents the interquartile range. Solid horizontal lines within boxes represent medians and dotted ones represent means. Tukey- style whiskers extend to maximum of one and half times of interquartile range beyond the boxes or maximal and minimal value without outliers. The dots denote outliers.

Abbreviations:

DCM: dilated cardiomyopathy; DCM-CHF: dilated cardiomyopathy with congestive heart failure; FAC: right ventricular fractional area change (%); RV: right ventricle; RVLS: right ventricular longitudinal strain of the right ventricular free wall (-1%); TAPSE: tricuspid annular plane systolic excursion (mm); TDI S': pulsed wave tissue Doppler imaging derived peak velocity of the systolic wave of the lateral tricuspid annulus (in cm/s). RV strain rate is -1/s.

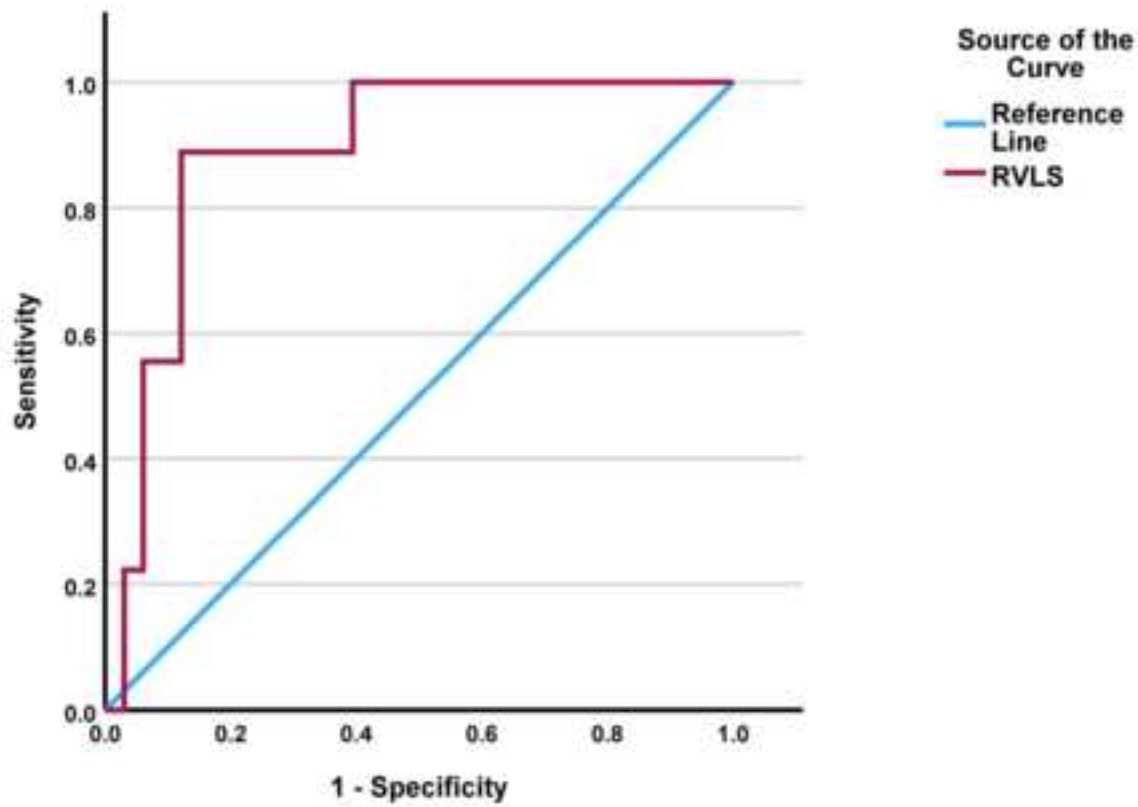
Figure 3

Receiver operator characteristic curve for right ventricular longitudinal systolic strain of the right ventricular free wall between normal and dogs with dilated cardiomyopathy with congestive heart failure which resulted in an AUC of 0.889 (confidence intervals: 0.783-0.995).

Abbreviations:

AUC:area under the curve; DCM: dilated cardiomyopathy; DCM-CHF: dilated cardiomyopathy with congestive heart failure; ROC: receiver operating characteristic curve; RVLS: right ventricular longitudinal strain of the right ventricular free wall (-1%)


Great Danes seen by Cardiology Service between January 2009- January 2023; n= 229 dogs
Equivocal echocardiographic results; n=86
Dogs excluded:
Heart tumor; n=1
Atrial fibrillation (AF) in the normal group (lone AF); n=1
No left sided views; n=4
Right heart not optimized from left apical views; n=1
Sustained ventricular tachycardia (VT) in the right sided views; n=2
Accelerated idioventricular rhythm (AIVR), n=1
Pacemaker (3rd degree atrioventricular block); n=1
Pulmonic stenosis/tricuspid dysplasia; n=1
Pulmonic stenosis; n=3
ASD/PFO=4
Systemic hypertension; n=2
Mitral dysplasia/pulmonary hypertension/congestive heart failure; n=1
Mitral dysplasia/Atrial fibrillation; n=1
Disseminated neoplasia/ascites/pleural effusion (suspected sarcoma) = 1
Inflammatory state (epididymitis, pyrexia); n=1
Theophylline=1
Pimobendan, furosemide, benazepril=1
Dogs included in the study; n=116 dogs
Dogs allocated to normal group; n= 74
Dogs allocated to PC- DCM group; n= 31
Dogs allocated to DCM/CHF group; n= 11


Sensitivity
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Reference Line
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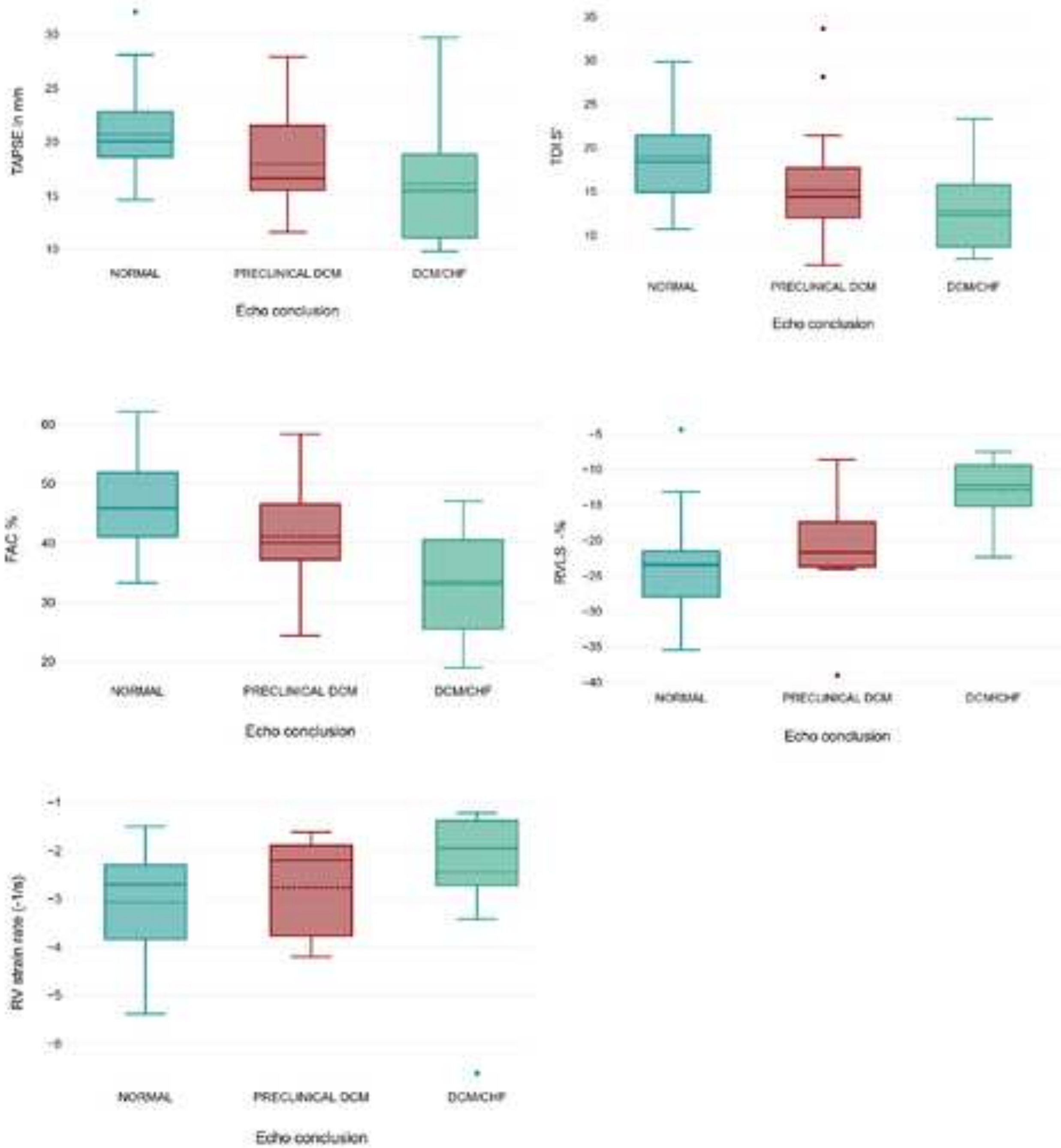
TAPSE in mm
NORMAL
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DCM/CHF
Echo conclusion
RVLS - %
NORMAL
PRECLINICAL DCM
DCM/CHF
Echo conclusion
RV strain rate (-1/s)
NORMAL
PRECLINICAL DCM
DCM/CHF
Echo conclusion

TABLE 1

Comparison of clinical and echocardiographic variables of all dogs. Values reported as median with 25th - 75th percentile.

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Body Weight (kg)	63.1 (57.93-68.68)		64.8 (53.3-91.1)		69 (55.5-95.6)		0.92
Age (years)	5.63 (4.25- 7.17)		6.67 (4-11.75)[a]		6.42 (2.83-10.08)[a]		**0.042**
TAPSE (mm)	20.05 (18.6-22.98)	51	16.6 (15.5-21.52) [a]	24	15.45 (11.08-18.83) [a]	10	**0.001**
TDI S' (cm/s)	18.4 (14.93 – 21.45)	74	14.4 (12-17.7) [a]	31	12.3 (8.7-15.75) [a]	11	**<0.001**
FAC (%)	45.8 (41.1-51.9)	41	40.05 (37.26-47.58)	20	33.05 (25.53-49.58) [ab]	10	**<0.001**
RVLS x (-1%)	23.33 (27.95-21.50)	26	21.71 (23.66-17.43)	7	12.19 (15.16-9.39) [ab]	9	**0.001**

a – p<0.05 compared to normal group

b – p<0.05 compared to pre-clinical group

Abbreviations:

DCM: dilated cardiomyopathy; DCM-CHF: dilated cardiomyopathy with congestive heart failure; FAC: right ventricular fractional area change; N: number of animals; PC-DCM: pre-clinical dilated cardiomyopathy; RV: right ventricle; RVLS: right ventricular longitudinal strain of the right ventricular free wall; TAPSE: tricuspid annular plane systolic excursion; TDI S’: pulsed wave tissue Doppler imaging derived peak velocity of the systolic wave of the lateral tricuspid annulus.

TABLE 2

Reference values for five echocardiographic indices of right ventricular systolic function assessed in 74 conscious healthy Great Danes (GDs).

RV function index	**Lower Reference interval**	***Lower 90%CI***	***Upper 90%CI***	**Upper Reference interval**	***Lower 90%CI***	***Upper 90%CI***
TAPSE mm	12.55	*11.04*	*14.23*	27.58	*25.63*	*29.60*
TDI S' cm/s	8.83	*7.49*	*10.10*	28.32	*26.53*	*30.31*
FAC %	30.92	*27.96*	*33.89*	60.57	*57.38*	*64.12*
RVLS x -1%	12.15	*9.04*	*14.66*	36.23	*33.21*	*40.26*
RVStrain rate x -1/s	0.524	*-0.11*	*0.96*	5.58	*4.97*	*6.58*

Abbreviations: CI: confidence intervals; FAC: right ventricular fractional area change; RV: right ventricle; RVLS: right ventricular longitudinal strain of the right ventricular free wall; TASPE: tricuspid annular plane systolic excursion; TDI S': pulsed wave tissue Doppler imaging derived peak velocity of the systolic wave of the lateral tricuspid annulus.

TABLE 3

Associations between right heart echocardiographic variables in 74 conscious healthy Great Danes with age, weight, and RR interval.

	age in years		**weight in kg**		**RR in ms**	
	r	P	r	P	r	P
TAPSE mm	0.01	0.938	0.1	0.471	-0.01	0.955
TDI S' cm/s	-0.06	0.603	-0.02	0.874	-0.18	0.148
FAC %	0.17	0.295	-0.14	0.392	-0.19	0.267
RVLS x-1%	-0.02	0.938	0.07	0.737	0.34	0.119
RV strain rate x-1/s	0.22	0.281	-0.06	0.786	0.54	**0.01**

Abbreviations:

FAC: RV fractional area change; $p < 0.05$, statistically significant; r: correlation coefficient; RV: right ventricle; RVLS: right ventricular longitudinal strain of the right ventricular free wall; TASPE: tricuspid annular plane systolic excursion; TDI S : pulsed wave tissue Doppler imaging derived peak velocity of the systolic wave of the lateral tricuspid annulus.

Table 4

Right ventricle echocardiographic variables: Receiver operator characteristic curve analysis to discriminate between echocardiographically normal Great Danes compared to those with dilated cardiomyopathy with congestive heart failure: selected cut-offs.

	AUC	95% CI AUC	Cut-off	SE%	SP%
TAPSE mm	0.737	0.543-0.932	<19.6	90	49.3
			<15.0	50	90.7
			<14.4	50	94.7
TDI S' cm/s	0.759	0.595-0.924	<18.3	90.9	41.9
			<11.8	45.5	91.4
			<13.8	63.6	81.0
FAC %	0.82	0.651-0.954	<44.6	90	47.5
			<35.9	60	90.2
			<34	60	93.4
RVSL x -1%	0.889	0.783-0.995	>22.4	100	60.6
			>13.3	55.6	90.9
			>16.3	88.9	87.9

In the table, for each variable, three cut-offs were selected which gave sensitivity (≥90%) (1st), specificity (≥90%) (2nd) and the cut-off with the highest Youden index (3rd) in detecting clinical DCM dogs.

Abbreviations:

AUC: area under the curve; CI: confidence interval; FAC: right ventricular fractional area change; RVLS: right ventricular longitudinal strain of the right ventricular free wall; SE: sensitivity; SP: specificity; TAPSE: tricuspid annular plane systolic excursion; TDI S': pulsed wave tissue Doppler imaging derived peak velocity of the systolic wave of the lateral tricuspid annulus.

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2. Drafting the work or revising it critically for important intellectual content; AND
3. Final approval of the version to be published; AND
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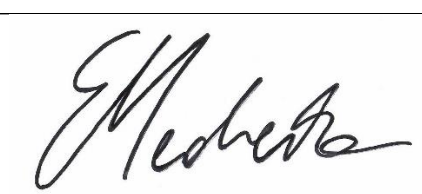
Please add signature here: 

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