Evaluation of right ventricular function using conventional and real-time three-dimensional echocardiography in normal dogs and dogs with myxomatous mitral valve disease

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Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

Master of Science

In

Biomedical & Veterinary Sciences

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April 27th, 2017

Blacksburg, Virginia

Keywords: Canine, Right Ventricle, Three-Dimensional, Echocardiography, Myxomatous Mitral Valve Disease

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ABSTRACT (Academic)

Objectives: To investigate the feasibility of real-time three-dimensional (3D) echocardiographic analysis of right ventricular (RV) function in healthy dogs and to compare conventional and 3D echocardiographic (3DE) indices of RV function in dogs with various stages of myxomatous mitral valve disease (MMVD), classified per the guidelines of the American College of Veterinary Internal Medicine, to those from healthy dogs.

Animals: Twenty-two healthy dogs and 56 dogs with MMVD

Methods: All dogs underwent conventional and 3D echocardiographic examinations. Measurements of 3DE RV function indices including RV end-diastolic volume (EDV), RV end-systolic volume (ESV), RV stroke volume (SV), and RV ejection fraction (EF) were recorded. Measurements of conventional indices of RV function were also obtained. RV EDV, ESV, and SV were indexed to bodyweight ($_{BW}$) and analyzed using commercially available software.

Results: Three-dimensional RV datasets could be acquired and analyzed in all dogs. Intra- and inter-observer coefficents of variation were > 20% for all 3D RV indices. Right ventricular EDV and ESV were decreased and RV EF was increased in dogs with advanced MMVD when compared to controls. Several conventional echocardiographic indices of RV function also differed between the control group and various MMVD groups.

Conclusions: Real-time 3DE RV assessment is feasible in normal dogs with acceptable intra- and inter-observer variability. Several 3DE indices of RV systolic function differ between dogs with advanced MMVD when compared to normal dogs. Further investigation is required to determine if these differences have clinical implications.

ABSTRACT (General Audience)

Background: Myxomatous mitral valve degeneration (MMVD) is the most common acquired heart disease in dogs. Real-time three-dimensional echocardiography (3DE) is a useful imaging modality for evaluation of right ventricular (RV) function in people with left-sided cardiac disease. The utility of 3DE evaluation RV function in dogs with MMVD has not been determined.

Objectives: To investigate the feasibility of 3DE analysis of RV function in healthy dogs and to compare conventional and 3DE indices of RV function in dogs with various stages of MMVD to those from healthy dogs.

Animals: Twenty-two healthy dogs and 56 dogs with MMVD

Methods: All dogs underwent conventional and 3D echocardiographic examinations and measurements of conventional and 3D indices of RV function were recorded. Three-dimensional volumetric data were indexed to bodyweight. Measurements were compared between normal dogs and dogs with various stages of MMVD.

Results: Three-dimensional RV datasets could be acquired and analyzed in all dogs. Within- and between-observer measurement variation was acceptable for all 3D RV indices. Right ventricular end-diastolic and end-systolic volumes were decreased and ejection fraction was increased in dogs with advanced MMVD when compared to controls. Several conventional echocardiographic indices of RV function also differed between the control group and dogs with various stages of MMVD.

Conclusions: Real-time 3DE RV assessment is feasible in normal dogs with acceptable intra- and inter-observer variability. Several 3DE indices of RV systolic function differ

between dogs with advanced MMVD when compared to normal dogs. Further investigation is required to determine if these differences have clinical implications.

DEDICATION

This thesis is dedicated to the memory of my loving aunt, Tolly.

Without her love, support and assistance, my career in the veterinary profession would not have been possible.

I also dedicate this work to my parents, Betty & Joe.

Their love, wisdom, support and encouragement have made me the man I am today.

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LIST OF ABBREVIATIONS

2D	Two-dimensional
3D	Three-dimensional
3DE	Three-dimensional echocardiography
ACVIM	American College of Veterinary Medicine
CMRI	Cardiac magnetic resonance imaging
CV	Coefficient of variation
EDA	End-diastolic area
EDA_{BW}	End-diastolic area indexed to bodyweight
EDV	End-diastolic volume
EDV_{BW}	End-diastolic volume indexed to bodyweight
EF	Ejection fraction
ESV	End-systolic volume
ESV_{BW}	End-systolic volume indexed to bodyweight
FAC	Fractional area change
PH	Pulmonary Hypertension
RV	Right ventricle / right ventricular
$RVDd^{base}$	Right ventricular diameter at end-diastole at the ventricular base / level of the tricuspid valve
$RVDd^{base}{}_{BW}$	Right ventricular diameter at end-diastole at the ventricular base / level of the tricuspid valve indexed to bodyweight
$RVDd^{mid}$	Right ventricular diameter at end-diastole at the level of the mid ventricle

$RVDd^{mid}_{BW}$	Right ventricular diameter at end-diastole at the level of the mid ventricle indexed to bodyweight
RVLd	Right ventricular length at end-diastole
$RVLd_{BW}$	Right ventricular length at end-diastole indexed to bodyweight
S’	Peak velocity of systolic mitral annular motion as determined by pulsed wave Doppler
SD	Standard deviation
SV	Stroke volume
SV_{BW}	Stroke volume indexed to bodyweight
TAPSE	Tricuspid annular plane systolic excursion
TEi	Myocardial performance index

PREFACE/ATTRIBUTION:

The studies presented herein were performed within the Department of Small Animal Clinical Sciences of the Virgina-Maryland College of Veterinary Medicine. Although all individuals who contributed to the projects are represented as authors, Dr. Aherne collected all data and authored all components of this thesis. The additional authors listed served roles in statistical analysis, the revision process, or the production of figures and tables.

1. Introduction & Literature Review

Right ventricular structure

The right ventricle (RV) of the heart is a complex geometric structure[1–4]. It is comprised of 3 distinct segments: the inlet, the trabecular apical myocardium, and the infundibulum[5–8]. The inlet extends from the tricuspid valve to the papillary muscles and, in addition to the afformentioned structures, it also contains the chordae tendineae[5,6]. As its name would suggest, the trabecular apical myocardium, extends from the papillary muscles to the right ventricular (RV) apex[5,6] and is characterized by many irregular ridges called trabeculae carneae[9,10]. The number of papillary muscles in the RV may vary[8,10]. The infundibulum, also termed the conus or right ventricular outflow tract (RVOT), is a region of smooth myocardium that extends from the RV apex to the pulmonary valve and is the RV outflow region[5,6].

Three muscular bands can be identified in the RV. These are the parietal band, the septomarginal band, and the moderator band[5,11]. An important identifying feature of the RV is the presence of a ventriculoinfundibular fold that separates the tricuspid and pulmonary valves as opposed to the fibrous continuity observed between the mitral and aortic valves in the left ventricle[5,8]. The septal insertion of ventriculoinfundibular fold and the parietal band together make up the crista supraventricularis[5,8], which is a stout, muscular structure projecting from the roof cranial to the tricuspid valve orifice[9]. The septomarginal band extends from the interventricular septum or base of the largest papillary muscle[12] and becomes continuous with the moderator band[9]. The septomarginal band provides a shortcut for a portion of the right branch of the atrioventricular bundle to allow for near complete coordination of contraction of all portions of the RV[9,12].

The right ventricle has a somewhat triangular shape when viewed sagitally[5,8,13] and appears crescenteric when viewed in transverse section as it wraps around the right, cranioventral aspect of the left ventricle[5,8,9,13]. The interventricular septum influences the shape of the right ventricle and under normal loading conditions is convex towards the right ventricle and concave towards the left ventricle in both systole and diastole[5].

The ventricular myocardium is a complex three-dimensional (3D) arrangement of myocytes in a fibrous tissue matrix[8]. The myocardium of the RV is comprised of superficial (subepicardial) and deep muscle layers. In the superfical layer, muscle fibers are arranged circumferentially in a direction parallel to the atrioventricular groove[5,8]. The arrangement of muscle fibers in the deep layer is predominantly longitudinal from apex to base[8,13], thus able to create a peristaltic contraction from the inlet to the infundibulum as well as a bellows-like motion of the free wall toward the septum[13].

There is little published data in the veterinary literature on the normal echocardiographic anatomy of the right ventricle in dogs. One recent study provided normal values for right atrial linear dimensions and areas, right ventricular internal linear dimensions and areas, right ventricular wall thicknesses and right ventricular outflow tract linear dimensions[14]. One group reported normal values for several two-dimensional (2D), m-mode and Doppler echocardiographic indices of RV systolic function but this study did not report normal RV linear dimensions or areas for their study population[15]. Another study compared 3D echocardiography (3DE) and cardiac computed tomography (CCT) to a reference standard of cardiac magnetic resonance imaging (CMRI) for evaluation of RV volumes in a cohort of healthy, anesthesized dogs[2]. This showed that both techniques were excellent correlated with CMRI but that 3DE underestimated

volumes and CCT overestimated volumes compared with CMRI. This study did not, however, analyze 2D echocardiographic (2DE) measurements of RV size.

Right Ventricular Function

The function of the RV is to receive blood from the systemic venous return and then pump it into the pulmonary arteries. Right ventricular contraction is a sequential process, beginning with contraction of the inlet and trabeculated apical myocardium and then ending with contraction of the infundibulum that occurs approximately 25 to 50 milleseconds later[5]. Right ventricular function is dependent on loading conditions *i.e.* preload and afterload. It can respond in different ways to changes in volume and pressure. The RV causes systolic flattening of the interventricular septum when faced with a pressure overload, which can also cause hypertrophy of the RV wall[7]. When volume overload is present, the RV becomes elongated and causes diastolic flattening of the interventricular septum[6]. The right ventricle dilates in response to chronic pressure or volume overload or RV failure[7].

Cardiac magnetic resonance imaging (CMRI) is considered the reference technique for the assessment of RV structure and function in people[2,16]. This technique, however, is limited by cost, availablity of equipment, availability of expertise, and, in the veterinary field, the requirement for general anesthesia. Various 2D, m-mode and Doppler echocardiographic techniques have been used to assess RV function in humans and animals. Standard 2DE assessment has several limitations. As a result of the complex geometry of the RV quantitative assessment of the RV by 2DE is difficult and RV size is significantly dependent on the angle of image visualization and acquisition[1,6,17]. Qualitative assessment may be inconsistent between observers[18]. Two-dimensional

assessment of the RV may be even less accurate in patients with dilation of the RV, which can be a common sequela to many pathological conditions[1]. Despite the inaccuracy of 2D echocardiography it remains the most widespread technique for the assessment of the RV in people[1–3,7]. Real-time 3DE is an evolving technology[6]. Volumetric analysis of the RV using 3DE have been shown to correlate well with measurements obtained by CMRI[2,4,6,16]. Real-time 3DE measurements tend to slightly underestimate RV volumes when compared to CMRI[2,4,17]. Current recommendations of the American Society of Echocardiography on evaluation of the RV are to measure the right atrial, RV and RVOT size using 2D echocardiography from standard acoustic windows and to evaluate RV systolic function using one or more of the following indices: 2D fractional area change (FAC), tricuspid annular plane systolic excursion (TAPSE) and peak velocity of the tricuspid annulus determined by pulsed-wave Doppler (S') and right ventrcular myocardial performance index (Tei)[19]. These recommendations also state that, where feasible, 3DE assessment of RV volumes and EF are recommended to complement the afforementioned 2DE measurements. Right ventricular 3D EF is representative of overall RV performance and provides and integrated assessment of the interaction between RV load and contractility[19].

There is limited data on the assessment of RV function in the veterinary literature. One study assessed normal values for 2D FAC, TAPSE, S' and 2D speckle-tracking-echocardiography (STE) derived strain and strain-rate of the RV free-wall[15]. This study found that all 3 indices were significantly associated with bodyweight and, by use of allometric scaling, reference intervals across a range of bodyweight were generated. A follow-up study by the same group assessed the effects of pimobendan and atenolol on

2D FAC, TAPSE, S' and RV 2D STE strain and strain rate[20]. In this study, pimobendan was found to cause an increase in all indices and, conversely, atenolol caused a decrease in all indices. Two-dimensionsal STE derived strain and strain rate of the RV demonstrated the highest percentage changes but also demonstrated the highest variability[20]. An afforementioned study on normal RV echocardiographic measurements also assessed the functional indices of FAC and TAPSE[14]. This study reported similar normal values for these variables compared to previously[15] but failed to identify a relationship between bodyweight and FAC[14]. As previously discussed, another study demonstrated an excellent correlation between both 3DE and CCT with CMRI in a small cohort of anesthetized beagles[2]. Right ventricular myocardial performance index (Tei) was investigated in 5 anesthetized Beagles and shown to be correlated with indices of RV function obtained by cardiac catheterization and also that it was independent of heart rate, bodyweight and age[21]. A study of 8 anesthetized, mixed breed dogs with experimentally induced biventricular congestive heart failure analyzed right ventricular function by means of gated radionuclide ventriculography[22]. This study found that RV EF was inversely related to pulmonary arterial systolic, diastolic and mean pressures and to pulmonary capillary wedge pressure. Right ventricular average emptying rate was also inversely related with pulmonary arterial systolic, diastolic and mean pressures. Right ventricular internal diastolic dimensions, determined by 2DE, were not associated with RV EF[22].

There are several studies reporting one or more of the afforementioned RV systolic function indices in dogs with various naturally-occuring cardiac diseases. Boxer dogs with arrhythmogenic right ventricular cardiomyopathy (ARVC) assessed by CMRI

had a significantly lower RV EF when compared with healthy size-matched hound dogs[23]. Another study of Boxer dogs with ventricular arrhythmias showed that TAPSE < 15.1 mm was associated with decreased survival time and was an independent predictor of cardiac death[24]. In a study of 30 dogs with pulmonary hypertension (PH) of various causes, TAPSE was shown to progressively decrease in association with increasing severity of PH when compared with a reference group of 50 healthy dogs[25]. Results from the reference group in this study also identified a significant association between TAPSE and bodyweight. A study of West Highland White Terriers with chronic pulmonary disease showed that 2D RV fractional shortening was reduced in dogs with pulmonary hypertension when compared to controls[26]. A retrospective study of 54 dogs, 41 of which had myxomatous mitral valve disease (MMVD) of varying severity assessed right heart size and shape by means of first pass radionuclide angiography[27]. The investigators determined that, in dogs with congestive heart failure (CHF) due to MMVD, the right heart chambers adopted a flattened appearance due to the dilated left chambers. They also concluded that the right heart chambers were enlarged, however it is important to note that the measurement technique used, FPRNA, allowed an assessment of area only and a relationship between area and volume cannot be assumed given the complex geometry of the RV[27]. A study of 70 Cavalier King Charles Spaniels with varying severity of MMVD had significantly lower TAPSE and S' when compared to a control population of 16 healthy Beagles[28]. A more recent study compared various tissue Doppler indices of right and left ventricular function, including S', in 86 dogs of various breeds with varying degrees of MMVD to results from 28 healthy dogs[29]. In this study, no association was identified between S' and the presence of MMVD or PH. However, dogs with CHF due

to MMVD did have increased early diastolic tricuspid annular velocities and dogs with stage B2 disease, defined as per the guidelines of the American College of Veterinary Internal Medicine (ACVIM), had increased late diastolic tricuspid annular velocities when both groups were compared with controls[29]. A study of RV myocardial performance index (Tei) in 86 dogs demonstrated that Tei was increased in dogs with tricuspid regurgitation (TR) and filariasis and, in the dogs with TR, Tei was increased in association with PH[21]. Right ventricular Tei has recently been shown to be a strong, independent predictor of cardiac death in a study of dogs with 30 dogs with MMVD[30].

Myxomatous Mitral Valve Disease

Myxomatous mitral valve disease is the most common acquired heart disease in dogs[31–36]. It is characterized by thickening and degeneration of the mitral valve leaflets resulting in regurgitation of blood from the left ventricle into the left atrium during systole. Affected valve leaflets shows an accumulation of proteoglycans and glycosaminoglycans with connective tissue derangements on histopathologic analysis[37]. In approximately 30% of cases, the tricuspid valve may also experience myxomatous degeneration[31]. Myxomatous mitral valve disease is age-related. The prevalence of the disease in older, small-breed dogs is 90-100%[35,38]. Small breeds are typically more commonly affected by MMVD, however larger breeds may also develop the disease[39,40]. The disease also has a higher prevalence in males versus females for any given age[39]. A long pre-clinical period characterizes MMVD in dogs[31,35], and many dogs with the disease do not develop CHF and die of causes other than heart disease[39]. In one study, over 70% of dogs with pre-clinical MMVD were still alive after a 6.6-year observation period and dogs with moderate or severe CHF had median survival times of 33 months and 9 months

respectively[41].

Myxomatous mitral valve disease is typically diagnosed and staged on the basis of physical examination findings and diagnostic imaging such as thoracic radiography and echocardiography. The American College of Veterinary Internal Medicine published guidelines for the diagnosis and treatment of the disease, in which, a staging system for MMVD was proposed[31] and is widely used[34]. Dogs with MMVD are divided into stages based on the results of clinical findings and diagnostic imaging. Dogs considered to be at a high risk of developing MMVD but currently have no identifiable structural cardiac disease are classified as Stage A. Dogs with pre-clinical MMVD are classified as Stage B. Stage B is further subdivided into two substages, B1 and B2. Stage B1 encompasses dogs with evidence of mitral regurgitation (MR) but without radiographic or echocardiographic evidence of left-sided chamber remodeling, while stage B2 dogs are dogs with evidence of MR and subsequent remodeling of the left heart chambers. Dogs with Stage C MMVD are those that have clinical signs as a result of their disease or previously had clinical signs that subsequently resolved with therapy. Dogs with CHF due to MMVD that is refractory to therapy are classified as Stage D.

Several risk factors have been identified in association with disease progression of MMVD. One of the most significant and most commonly encountered risk factors for progression is left atrial enlargement[32,36,41,42]. A common complication of MMVD encountered in dogs is pulmonary hypertension (PH)[38,43,44]. In humans, PH is identified in approximately 60-80% of patients with left-sided heart disease[43]. The prevalence of PH in dogs varies with values of 14-53% reported[43,44]. In a retrospective study of 212 dogs with ACVIM stage B2 and stage C MMVD the prevalence of PH was 39%[38]. In this

study, PH was more commonly identified in stage C dogs versus stage B2 dogs. Left atrial size indexed to aortic diameter and severity of PH based on the tricuspid regurgitation pressure gradient were significant negative prognostic indicators[38]. Initially, PH attributable to MMVD is the result of passive back transmission of elevated left heart filling pressures to the pulmonary capillaries[38,43]. However, sustained elevation of pulmonary venous pressures and acute or chronic hypoxia can lead to reactive vasoconstriction of the pulmonary arterioles and irreversible remodeling[38,43]. In patients with left-sided heart disease, the degree of PH is partially dependent on RV performance[43].

Evaluation of RV ventricular function is an important prognostic and decision-making tool in people with MMVD[6,18,45,46]. Chronic left-sided CHF is the most common cause of RV dysfunction in people[47]. In these patients, RV dysfunction may result from PH, the inherent interdependence of the ventricles, intrinsic myocardial involvement, neurohormonal pathways or myocardial ischemia[47]. Right ventricular EF is a strong, independent predictor of death in humans with left-sided heart failure[47]. As previously discussed, there are comparatively few studies in the veterinary literature investigating RV function in canine MMVD[25,27–30]. To the author's knowledge there are, to date, no studies evaluating 3DE assessment of RV function in dogs with MMVD.

Objectives and Hypotheses

We designed a prospective, two-phased study to evaluate RV function using 3DE and conventional (2D, m-mode and Doppler) echocardiography in conscious, healthy dogs and in conscious dogs with MMVD. The aims of the 1st study were: a) to prospectively assess the feasibility of 3DE analysis of RV function in a population of

conscious, unsedated, healthy dogs and b) to report the findings of 3DE and conventional (2D, m-mode and Doppler) echocardiographic indices of RV function in these dogs. We hypothesized that: a) acquisition and measurement of 3DE indices of RV function would be feasible in conscious, unsedated dogs, and b) measurement of 3DE indices of RV function would have good repeatability.

For the 2nd study, our aims were: a) to prospectively measure conventional (2D, m-mode, Doppler) echocardiographic and 3DE indices of RV systolic function in dogs with various stages of MMVD, as defined by the guidelines of the ACVIM, and b) to compare the measurements of conventional and 3DE indices of RV function in dogs with different stages of MMVD to those obtained from normal, healthy dogs. Our hypotheses were: a) that indices of RV systolic function would be reduced in dogs with MMVD when compared with normal, healthy, controls, and b) that the degree of systolic dysfunction would be correlated with the clinical stage of MMVD.

Materials and Methods

For our 1st study, healthy dogs volunteered by staff and students of the Virginia-Maryland College of Veterinary Medicine (VMCVM) were prospectively enrolled. Two-dimensional, m-mode, Doppler and real-time 3D echocardiographic RV datasets were acquired from all dogs without sedation. Datasets were analyzed with commercially available software. A semi-automated procedure was used to generate a 3D model of the RV allowing RV indices to be calculated: end-diastolic volume (EDV); end-systolic volume (ESV); stroke volume (SV), and ejection fraction (EF). Intra- and inter-observer coefficients of variation were calculated for 3D EDV, 3D ESV and 3D SV. Associations between RV volumes and size were evaluated and the datasets were then used to generate

reference intervals. Full details on materials, methods and results of this study are provided in Section 2: Evaluation of right ventricular function using conventional and real-time three-dimensional echocardiography in normal dogs.

For our 2nd study we prospectively enrolled the cohort of healthy dogs described in the 1st study and client-owned dogs presenting to the cardiology service of the Veterinary Teaching Hospital of the VMCVM with a confirmed diagnosis of MMVD. Dogs with MMVD were staged in accordance with ACVIM guidelines[31]. All dogs underwent conventional (2D, m-mode and Doppler) and 3D echocardiography. Conventional and 3D echocardiographic indices of RV systolic function including RV end-diastolic volume (EDV), RV end-systolic volume (ESV), RV stroke volume (SV), RV ejection fraction (EF) were recorded. Measurements of conventional (2D, m-mode and Doppler) indices of RV function were also obtained. Based on the findings of our 1st study, RV EDV, ESV, SV, 2D linear dimensions and 2D areas were indexed to bodyweight. Data were analyzed using commercially available software. Non-parametric data were assessed using a Kruskal-Wallis test with subsequent post-hoc analysis using Dunn's method for multiple comparisons between groups. A P-value of <0.05 was considered significant. Full details on the materials, methods and results of this study are provided in Section 3: Evaluation of right ventricular function using conventional and real-time three-dimensional echocardiography in dogs with myxomatous mitral valve disease.

1. Evaluation of right ventricular function using conventional and real-time three-dimensional echocardiography in normal dogs

a. Title: Evaluation of right ventricular function using conventional and real-time three dimensional echocardiography in normal dogs

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Short title for running head: Evaluation of RV function using 3D echo in normal dogs

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Acknowledgments: The authors would like to thank the faculty, staff and students of the Virginia-Maryland College of Veterinary Medicine who volunteered their pets for this study as well as Dr. Sandra Müller and Dr. Raelyn Walczak for their technical assistance.

b. Abstract

Objectives: To investigate the feasibility of real-time three-dimensional echocardiographic (3DE) analysis of the right ventricle and to report the findings of 3DE indices of right ventricular (RV) function in healthy, conscious, unsedated dogs

Animals: Twenty-two normal, healthy dogs

Methods: Two dimensional (2D), m-mode, Doppler and real-time 3D echocardiographic RV datasets were acquired from healthy, unsedated dogs. Datasets were analyzed with commercially available software. A semi-automated procedure was used to generate a 3D model of the RV and 3D end-diastolic volume (EDV), 3D end-systolic volume (ESV), 3D stroke volume (SV) and 3D ejection fraction (EF) were automatically computed. Intra- and inter-observer coefficients of variation were calculated for 3D EDV, 3D ESV and 3D SV. Associations between RV volumes and body size were evaluated and prediction intervals were generated.

Results: 3D RV datasets could be acquired and analyzed in all dogs. Intra- and interobserver coefficients of variation were less than 20% for all 3D RV indices. As determined by coefficients of determination (R^2), the goodness of fit was highest between $\log^{10}$ transformed RV variables and $\log^{10}$ bodyweight for all 2D and 3D RV echocardiographic variables.

Conclusions: Real-time 3D echocardiography of the right ventricle is feasible in normal, conscious, unsedated dogs. 3D RV volume measurements can be indexed to bodyweight on the basis of an allometric relationship. Prediction intervals for RV volume measurements are proposed across a variety of bodyweights.

Key words: canine, heart, diagnostic imaging

Abbreviations:

2D	Two-dimensional
3D	Three-dimensional
3DE	Three-dimensional echocardiography
CMRI	Cardiac magnetic resonance imaging
CV	Coefficient of variation
EDV	End-diastolic volume
EF	Ejection fraction
ESV	End-systolic volume
FAC	Fractional area change
RV	Right ventricular
$RVDd^{base}$	Right ventricular diameter at end-diastole at the ventricular base / level of the tricuspid valve
$RVDd^{mid}$	Right ventricular diameter at end-diastole at the level of the mid ventricle
RVLd	Right ventricular length at end-diastole
S’	Peak velocity of systolic mitral annular motion as determined by pulsed wave Doppler
SD	Standard deviation
SV	Stroke volume
TAPSE	Tricuspid annular plane systolic excursion

c. Introduction

Understanding of right ventricular (RV) function is relatively limited. For many years, RV function was largely overlooked in both human[1–4] and veterinary medicine[5]. In recent years, however, the evaluation of RV function in people with a variety of cardiac and non-cardiac conditions has received increased attention and has become an important tool in prognostication and decision-making[6–9]. The complex shape and geometry of the right ventricle has made accurate quantification and evaluation of RV function challenging[5,8–10]. Various indices of RV function using 2-dimensional (2D) and M-mode echocardiography have been employed including, but not limited to, fractional area change (FAC), speckle-tracking echocardiography (STE)-derived strain, STE-derived strain rate, tricuspid annular plane systolic excursion (TAPSE) and tissue Doppler imaging-derived systolic myocardial velocity of the lateral tricuspid annulus (S')[5].

Cardiac magnetic resonance imaging (CMRI) has been considered the gold-standard modality for the assessment of RV structure and function[4,9,11]. However, in a veterinary setting, CMRI has limitations that include the requirement for general anesthesia, cost and the limited availability of the necessary equipment and expertise. Other modalities including gated radionuclide ventriculography[12], cardiac computed tomography and 3-dimensional echocardiography (3DE)[11] have also been used to evaluate RV volumes. One recent study showed excellent agreement between 3D echocardiography and CMRI for assessment of RV volume and function in anesthetized, experimental dogs[11], a finding in agreement with several studies in people[7–9].

To date, there have been very few studies of RV function in dogs and there is very limited data evaluating RV function in dogs with acquired heart disease[13–15]. To the

authors' knowledge, there have been no studies evaluating RV function using 3D echocardiography in conscious, healthy dogs. Our aims were to assess the feasibility of 3D echocardiographic analysis of the right ventricle and report the findings of 3D echocardiographic indices of RV function in a cohort of healthy, unsedated dogs.

d. Animals, Materials and Methods

Animals

Staff-, student- and client-owned dogs presented to the cardiology service at the Virginia-Maryland College of Veterinary Medicine's teaching hospital were prospectively enrolled between June 2015 and February 2016. Dogs that were deemed healthy, based on history, physical examination and conventional (2D, M-mode, and Doppler) echocardiography were included. Dogs weighing < 5kg or > 25 kg, dogs with evidence of non-cardiac systemic disease, and dogs with heart murmurs of intensity greater than grade II/VI were excluded prior to conventional echocardiography. Boxers and English Bulldogs were excluded due to the risk of occult arrhythmogenic right ventricular cardiomyopathy in these breeds[16,17]. Dogs with echocardiographic evidence of congenital or acquired cardiac disease were excluded. Dogs requiring sedation to facilitate echocardiography were also excluded. This study was approved by the Institutional Animal Care and Use Committee of Virginia Tech (IACUC # - 15-061).

Echocardiographic examination

Conventional (2D, M-mode and Doppler) Echocardiography

All echocardiographic studies were performed by a single operator (MA) using an Artida echocardiographic system[a] with simultaneous ECG recording. Transducer selection was matched to patient size and preset for image optimization. Standard echocardiographic planes directed from right and left windows were obtained with the dogs gently restrained in right and left lateral recumbency respectively[18]. Left-apical 4-chamber views optimized for the right-heart were used for assessment of end-diastolic RV diameter at the ventricular base / level of the tricuspid valve ($RVDd^{base}$), end-diastolic RV diameter at the mid-ventricle ($RVDd^{mid}$), right ventricular end-diastolic length (RVLd), RV end-diastolic area (EDA), RV end-systolic area (ESA), RV fractional area change (FAC), tricuspid annular plane systolic excursion (TAPSE) and peak velocity of systolic tricuspid annular motion as determined by pulsed wave Doppler (S') as previously described[5,19–21]. All data were digitally captured for later off-line analysis on a digital workstation[b]. An average of at least three, usually consecutive, representative measurements was recorded for each variable.

Real-time three-dimensional echocardiography

Dogs without evidence of structural heart disease on conventional echocardiography underwent the following specific echocardiographic examination, as a continuation of the initial echocardiogram. Real-time 3DE datasets were acquired using a full matrix array transducer with 3D functionality[c] directed from a left-apical acoustic

window. Alignment was guided by a multi-plane real-time view (Pre-4D; Fig. 1), comprised of two orthogonal long-axis views and three short-axis views with the transducer positioned such that the orthogonal long-axis views were a standard left-apical four-chamber view and a standard left-apical two-chamber view. Depth, azimuth and elevation were adjusted to ensure the entire right ventricle was included. 3DE datasets were then acquired with the acquisition modality in full 4D mode, using data from four consecutive heartbeats. A minimum of three consecutive datasets from each patient was stored for later off-line analysis.

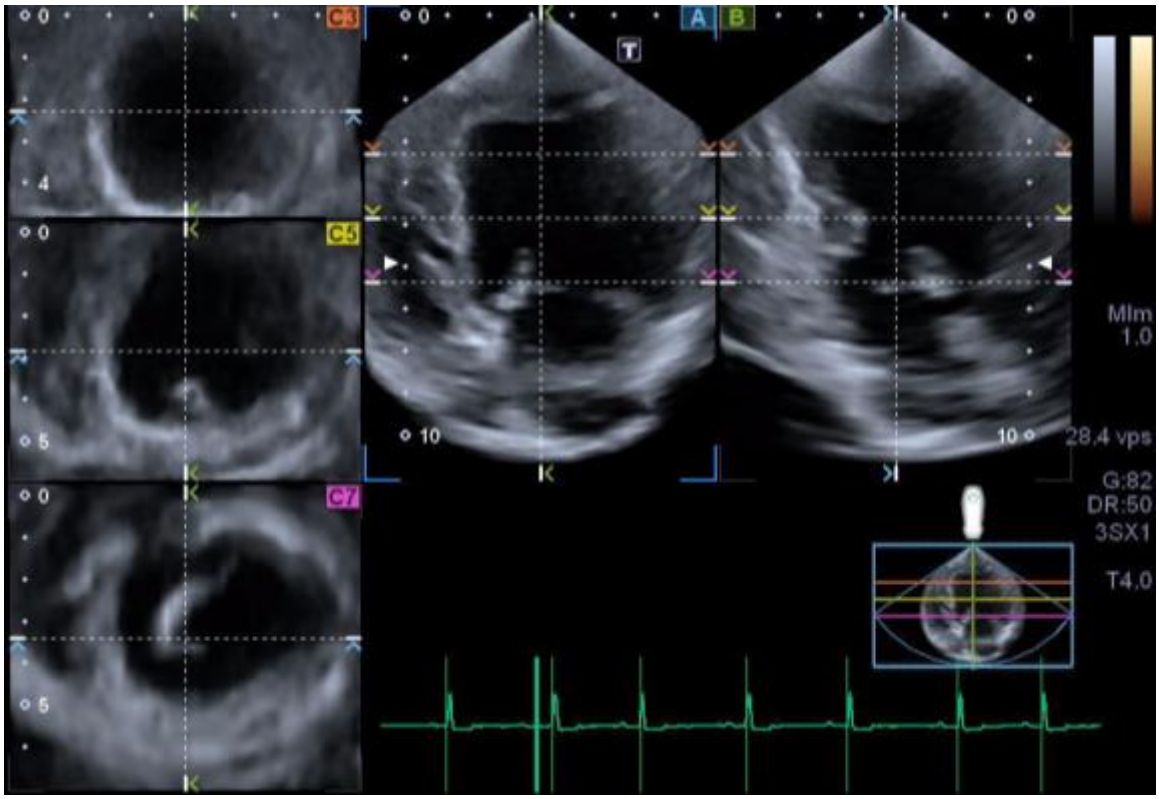

Figure 1. Pre-4D multi-plane image: During acquisition of 3D datasets, the ultrasound probe is directed so that two orthogonal long-axis views corresponding to the left-apical 4-chamber view optimized for inclusion of the right ventricle (labeled A on the ultrasound image) and left apical 2-chamber view (labeled B) were displayed. Three simultaneous short-axis images are also displayed (labelled C3 [orange], C5 [yellow] and C7 [pink]) and correspond to planes identified on the long axis images denoted by arrows of the same color respectively.

Image analysis

All conventional and 3D echocardiographic studies were analyzed off-line by a single observer (MA) using workstations equipped with dedicated software packages[b,d]. 3DE datasets were imported and a semi-automated procedure was used to generate a model of the RV. The center of the mitral valve and the left ventricular apex were identified and labeled in orthogonal apical four- and two-chamber long-axis views focused on the left ventricle. The center of the tricuspid valve and the RV apex were then identified and labeled in orthogonal apical four- and two- chamber views focused on the right ventricle. The aortic valve annulus was identified and labeled in an apical five-chamber view. Finally, a short-axis view of the right ventricle was used to identify and label the endocardium of both the interventricular septum and the RV free-wall as well as the endocardium at the points of the crescent shape formed by the right ventricular lumen in a short-axis view (Fig. 2).

The datasets were manually reviewed to ensure correct tracking of the endocardium by the software in both systole and diastole and to correct any tracking errors. The final 3D RV model, termed a 'beutal' (Fig. 3.), was then generated and the following measurements were automatically measured: 3D end-diastolic volume (EDV), 3D end-systolic volume (ESV), 3D stroke volume (SV) and 3D ejection fraction (EF). Ten datasets were randomly selected using a random sequence generator. These datasets were analyzed by two different observers (MA and GM), each on two separate occasions, at least 1 day apart, in order to determine intra- and inter-observer variability. Both observers were blinded to the results of previous analyses and analyses performed by the other observer.

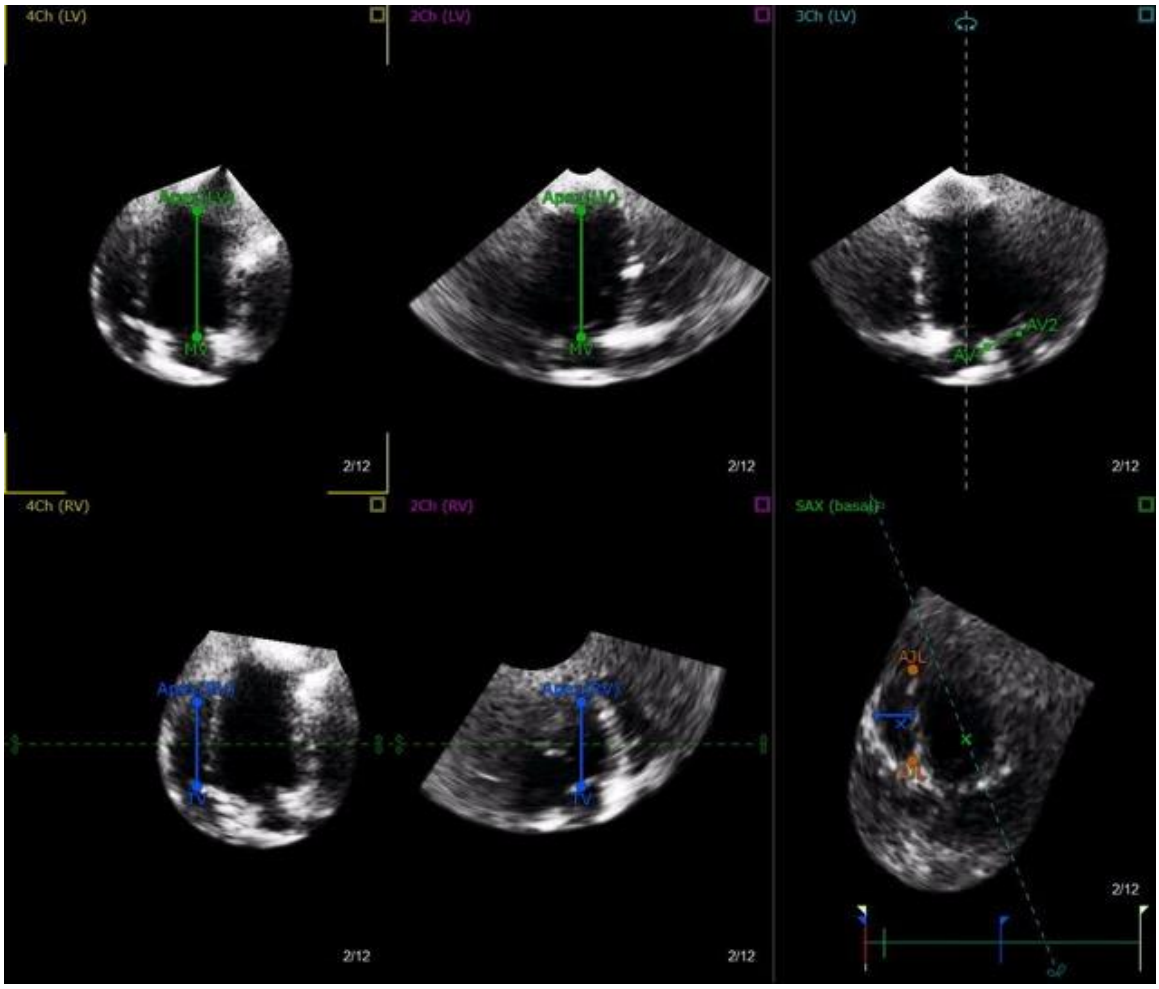

Figure 2. **Image adjustment:** Images corresponding to the left-apical 4-chamber view and left-apical 2-chamber view are identified and a line is placed extending from the coaptation point of the mitral valve to the left ventricular apex (green lines). On two orthogonal views of the right ventricle, a line is then placed extending from the coaptation point of the tricuspid vale to the right ventricular apex (blue lines). On an image corresponding to a left-apical 5-chamber view (reversed), a line is drawn connecting the hinge points of the aortic valve (upper right image). Finally, on an image corresponding to a right parasternal short-axis image of the ventricles, two markers (orange dots) are placed at the points of the 'crescent' formed by the right ventricle and a line is drawn extending from the endocardial border of the right ventricular septum to endocardial border of the right ventricular free wall.

Data analysis

Data were analyzed using a commercially available software package[e]. Normality was visually assessed using normal quantile plots. Homoscedascity was assessed by means of Levene's test. Normally distributed data are reported as mean ± standard

deviation (SD). Non-normally distributed data are reported as median (range: min-max). For each of the ten randomly selected datasets, within- and between-observer % coefficients of variation (CV%) were calculated for each 3D RV measurement and expressed as a percentage using the equation: CV% = (SD of measurements / average of measurements) x 100. From these values, the mean CV% ± SD was then calculated for each variable.

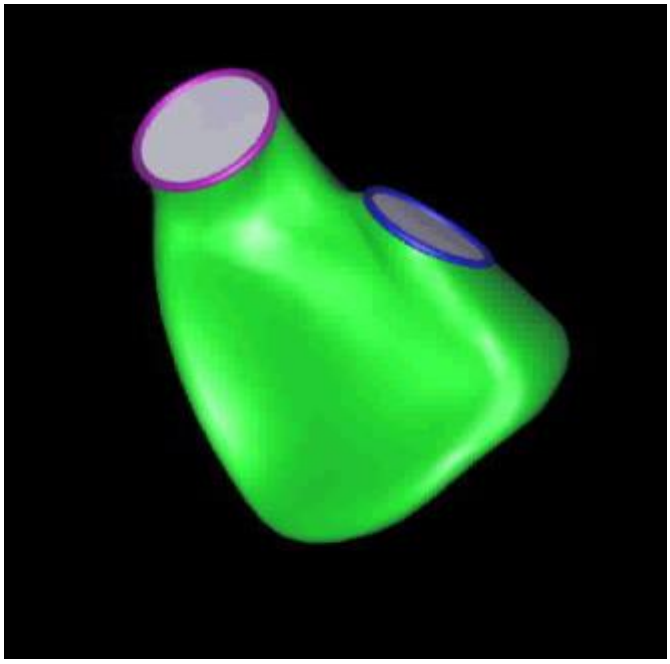

Figure 3. Standard three-dimensional right ventricular beutal: The blue ring corresponds to the tricuspid annulus. The pink ring corresponds to the pulmonic annulus.

It was assumed a relationship existed between 3D RV dimensions and body size. In order to identify the most suitable measure of body size with which to index RV echocardiographic variables, exploratory analyses were performed. Briefly, simple linear regression was used to evaluate relationships between echocardiographic RV variables and bodyweight and between echocardiographic RV variables and body surface area. Log^{10} transformed echocardiographic RV variables were regressed against log^{10} of

bodyweight in order to investigate the possibility of an allometric relationship between RV dimensions and body size. The best goodness of fit, as determined by the coefficient of determination (R^2), was identified between log^{10} transformed RV echocardiographic variables and log^{10} bodyweight. For each variable, the terms of the allometric equation $y=ax^b$ were then defined from the regression as follows: the scaling exponent (b) is the slope of the regression line; the proportionality constant (a) is the antilog (log^{-1}) of the intercept, and x is bodyweight[22]. Predicted values and 95% prediction intervals were generated across a range of bodyweights for 3D RV variables.

e. Results

Thirty-two dogs were initially presented for assessment with twenty-two dogs suitable for inclusion. Three dogs were excluded prior to echocardiography; two of these dogs had murmurs greater than grade II/VI intensity and one dog weighed less than 5 kg. Seven dogs were excluded due to evidence of myxomatous mitral valve disease on conventional echocardiography.

Table 1. Age, sex, bodyweight, body surface area and heart rate

Variable	Value
Sex (M/F)	10/12
Age (y)	4.49 (range: 0.86-11.39)
Bodyweight (kg)	15.75 (range: 6-25)
Body surface area (m^2)	0.64 (range 0.33-0.86)
Heart rate (bpm)	110.3 ± 22.7

Descriptive statistics for sex, age, bodyweight, body surface area and heart rate are shown in table 1. Median age was 4.49 years (range: 0.86-11.39). Median bodyweight was 15.75 kg (range: 6.00-25.00). Ten males and twelve females were represented. Four of the 22 dogs (18.18%) had innocent murmurs. Breeds represented included 12 mixed breeds, 3 Staffordshire Terriers and 1 each of the following: Australian Shepherd, Brittany Spaniel, Canaan Dog, Golden Retriever, Labrador Retriever, Pembroke Welsh Corgi and Shih Tzu.

Table 2. Two-Dimensional, M-mode and Doppler Echocardiographic Right Ventricular Measurements

Variable	Mean / Median (n=22)
S' (m/s)	0.11 (range: 0.07-0.19)
TAPSE (mm)	13.7 (range: 10.2-15.6)
End-diastolic Area (cm^2)	6.82 ± 2.34
End-systolic Area (cm^2)	3.74 ± 1.41
FAC (%)	46.65 (range: 25.5-58.5)
$RVDd^{base}$ (mm)	15.95 (range: 9.8-21.3)
$RVDd^{mid}$ (mm)	19.95 ± 4.07
RVLd (mm)	37.15 ± 5.77

S', peak velocity of systolic mitral annular motion as determined by pulsed wave Doppler; TAPSE, tricuspid annular plane systolic excursion; FAC, fractional area change, $RVDd^{base}$, right ventricular diameter at end-diastole taken at the level of the tricuspid annulus; $RVDd^{mid}$, right ventricular diameter at end-diastole taken at the level of the mid-ventricle; RVLd, right ventricular length at end-diastole.

Descriptive statistics for 2D, M-mode and Doppler echocardiographic indices of right ventricular function are presented in table 2. All log transformed RV linear and area

measurements were significantly associated with bodyweight. Coefficients of determination for 2D end-diastolic area ($p < 0.0001$), 2D end-systolic area ($p < 0.0001$), $RVDd^{base}$, $RVDd^{mid}$ and RVLd were 0.81, 0.74, 0.79 ($p < 0.0001$), 0.65 ($p = 0.0002$) and 0.79 ($p < 0.0001$) respectively. Fractional area change, TAPSE, S' and 3D EF all shared no significant associations with body size. Predicted values and prediction intervals for 2D, M-mode and Doppler echocardiographic measurements of RV size and function have been previously described[5,19].

Table 3. Mean values, inter-observer and intraobserver measurement variability for three-dimensional indices of right ventricular function

Variable	Value (Mean ± SD)	Inter-observer CV%	Intra-observer CV%
3D EDV (ml)	18.20 ± 8.31	15.2	12.3
3D ESV (ml)	11.39 ± 5.31	18.0	9.6
3D SV (ml)	6.76 ± 3.14	15.2	16.0
3D EF (%)	37.65 ± 4.65	12.0	9.0

3D EDV, Three-dimensional end-diastolic volume; 3D ESV, Three-dimensional end-systolic volume; 3D SV, Three-dimensional stroke volume; SD, standard deviation; CV%, percentage coefficient of variation.

Acquisition and subsequent analysis of 3D RV datasets was possible in all dogs. Table 3 shows descriptive statistics of 3D indices of RV function and intra- and inter-observer coefficients of variation (CV) for these indices. Intra- and inter-observer CV% were less than 20% for all 3DE RV indices. Log transformed 3D volume measurements were all significantly associated with bodyweight. Coefficients of determination for 3D EDV, 3D ESV and 3D SV were 0.7 ($p < 0.0001$), 0.69 ($p < 0.0001$) and 0.67 ($p < 0.0001$)

respectively. Table 4 presents predicted values and 95% prediction intervals for 3D RV volumes over a range of bodyweights.

Table 4. Predicted values and 95% prediction intervals for three-dimensional volumetric measurements

Bodyweight (kg)	3D EDV (ml)	3D ESV (ml)	3D SV (ml)
5	4.34 (2.01-9.35)	2.57 (1.13-5.85)	1.72 (0.79-3.77)
7	6.44 (3.15-13.1)	3.87 (1.81-8.31)	2.52 (1.22-5.19)
9	8.66 (4.36-17.17)	5.26 (2.53-10.93)	3.33 (1.66-6.67)
11	10.96 (5.61-21.39)	6.71 (3.28-13.71)	4.17 (2.12-8.21)
13	13.34 (6.89-25.84)	8.22 (4.06-16.66)	5.02 (2.57-9.81)
15	15.78 (8.17-30.5)	9.79 (4.84-19.78)	5.89 (3.02-11.48)
17	18.29 (9.45-35.38)	11.39 (5.63-23.06)	6.77 (3.47-13.22)
19	20.84 (10.73-40.47)	13.04 (6.42-26.50)	7.67 (3.92-15.02)
21	23.44 (12.01-45.76)	14.73 (7.21-30.10)	8.58 (4.36-16.89)
23	26.09 (13.28-51.25)	16.46 (8.00-33.84)	9.49 (4.79-18.81)
25	28.78 (14.55-56.93)	18.21 (8.79-37.74)	10.42 (5.22-20.80)
Proportionality Constant (a)	0.653	0.364	0.286
Scaling Exponent (b)	1.176	1.216	1.116

3D EDV, Three-dimensional end-diastolic volume; 3D ESV, Three-dimensional end-systolic volume; 3D SV, Three-dimensional stroke volume.

f. Discussion

The results of this study demonstrate that transthoracic real-time 3D evaluation of the right ventricle is feasible in healthy, conscious, unsedated dogs. The feasibility of

acquisition of 3D RV datasets and the feasibility of off-line analysis of these datasets were both 100%. These high feasibilities are likely attributable to the experience of our group with this imaging modality in prior studies[23,24] which highlights the learning curve encountered in utilizing this imaging technique.

Prior to this study, the feasibility of 3D echocardiographic evaluation of RV function had only been evaluated in anesthetized dogs where it was demonstrated to have good-to-excellent correlation with CMRI; however, it slightly underestimated RV volumes when compared to CMRI[11]. Cardiac MRI is considered to provide the most accurate assessment of RV structure and function in both humans and canines[4,11,25]. CMRI is inherently limited in veterinary medicine due factors such as cost, availability of equipment, availability of experienced personnel, time for data acquisition and the requirement for general anesthesia. Our results show that real-time 3D echocardiography is a feasible method for RV assessment in conscious dogs. Favorably, it negates the requirement for general anesthesia, which is of particular importance in patients with existing impairment to cardiac function.

The results of linear RV measurements obtained from 2D echocardiography in the present study are similar to recently published data[19]. As would be expected, linear, area and volumetric indices were all significantly associated with body size. With regards 3D RV volumetric data, the results of 3D ESV in this study are similar to those reported previously[11]. Three-dimensional EDV, 3D SV and 3D EF in the present study were all lower than values previously reported by Sieslak et al[11]. These differences may possibly reflect differences between study population, differences between operators across studies or the effects of general anesthesia the canine right ventricle[11,26,27].

Our results for FAC are concordant with the findings of other studies[5,19]. Similar to the findings of Gentile-Solomon and Abbott[19], no association was identified between FAC and body size, which is in contrast with the findings of Visser et al[5]. It is possible that our sample size was insufficient to detect a small effect; however, the fact that FAC is a dimensionless measure should be considered. Since FAC is a ratio of 2 area measurements, it would logically follow that it would maintain a constant value amongst animals with similar geometry but of varying size.

Measurements of S' in this study are similar to results obtained in prior studies[5,21]. Similar to Chetboul et al[21], we failed to find any association between S' and body size. This is in contrast with the findings of Visser et al[5]. The measurements of TAPSE, in this present study, are also in agreement with the results of other studies[5,19,20]. However, unlike these studies we did not identify an association between TAPSE and body size. A possible explanation for the lack of associations between S' or TAPSE and body size is our small sample size, which may not have allowed detection of small effects.

Limitations

This study is primarily limited by the small sample size. A larger population would be required to generate more accurate and precise reference intervals. The prediction intervals presented are derived only for the range of bodyweights encountered. Interpretation of values from patients outside this weight range should be interpreted with caution. It is also possible that a larger sample would have allowed detection of small effects between body size and various indices of RV function as have been previously described[5,19,20].

The cross-sectional nature of the study is another limitation. Longitudinal follow-up was not performed and within-subject variability was not examined in this study, so any potential changes in measurement variables over time cannot be evaluated. Despite attempts to exclude dogs with a higher risk of occult right ventricular dysfunction[16,17], we cannot guarantee that dogs included in the study were free of subclinical cardiomyopathies that could have affected results.

Additionally, all datasets were acquired by a single operator. The effect of intra-operator variability during acquisition of datasets on measurements of 3D RV function was not examined in this study.

Conclusion

Three-dimensional echocardiographic evaluation of RV function is feasible in conscious, healthy dogs, with acceptable intra- and inter-observer coefficients of variation for 3DE indices of RV function. Further studies are warranted to investigate for changes in these variables in normal dogs over time and to investigate the utility of 3DE indices of RV function in dogs with clinical cardiac disease.

Footnotes

a. Artida, Toshiba Medical Systems, Tokyo, Japan
b. Image Arena, TomTec Imaging Systems, Unterschleissheim, Germany
c. PST-25SX matrix array transducer, Toshiba Medical Systems, Tokyo, Japan
d. 4D RV-Function, TomTec Imaging Systems, Unterschleissheim Germany
e. JMP Pro 12, SAS Institute Inc., Cary, N.C., USA

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2. Evaluation of right ventricular function using conventional and real-time three-dimensional echocardiography in normal dogs

a. Title: Evaluation of right ventricular function using conventional and real-time three-dimensional echocardiography in dogs with myxomatous mitral valve disease

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Short title for running head: Evaluation of RV function using 3D echo in dogs with MMVD

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Acknowledgments: The authors would like to thank the faculty, staff and students of the Virginia-Maryland College of Veterinary Medicine (VMCVM) and the clients of the cardiology service of the Veterinary Teaching Hospital of the VMCVM who volunteered their pets for this study. The authors would also like to thank Dr. Sandra Müller and Dr. Raelyn Walczak for their technical assistance.

a. Abstract

Objectives: To compare conventional and 3D echocardiographic indices of right ventricular (RV) systolic function in dogs with various stages of myxomatous mitral valve disease (MMVD), classified according to the guidelines of the American College of Veterinary Internal Medicine (ACVIM), to those from normal dogs.

Animals: 78 unsedated dogs (22 healthy controls, 23 ACVIM Stage B1 MMVD, 20 ACVIM Stage B2 MMVD, 13 ACVIM Stage C MMVD)

Methods: All dogs underwent conventional and 3D echocardiography. 3D RV end-diastolic volume (EDV), end-systolic volume (ESV), stroke volume (SV) and ejection fraction (EF) were recorded. RV EDV, ESV, and SV were indexed to bodyweight ($_{BW}$). Non-parametric data were assessed using a Kruskal-Wallis test with subsequent post-hoc analysis using Dunn's method for multiple comparisons between groups. A P-value of <0.05 was considered significant.

Results: RV EDV_{BW} was decreased in stage B1 ($p < 0.05$), stage B2 ($p < 0.05$) and stage C ($p < 0.01$) dogs when compared to controls. ESV_{BW} was decreased stage B2 ($p < 0.01$) and stage C ($p < 0.001$) dogs when compared to controls. SV_{BW} was lower in stage B2 dogs when compared to controls ($p < 0.01$). EF was higher in stage C dogs compared to controls ($p < 0.01$) and to stage B1 ($p < 0.01$) dogs.

Conclusions: Several 3D echocardiographic indices of RV systolic function differ between dogs with advanced MMVD when compared to normal dogs. Further investigation is required to determine if these differences have clinical implications.

Key words: canine, heart, degeneration, diagnostic imaging

Abbreviations:

2D	Two-dimensional
3D	Three-dimensional
3DE	Three-dimensional echocardiography
ACVIM	American College of Veterinary Medicine
CMRI	Cardiac magnetic resonance imaging
CV	Coefficient of variation
EDA	End-diastolic area
EDA_{BW}	End-diastolic area indexed to bodyweight
EDV	End-diastolic volume
EDV_{BW}	End-diastolic volume indexed to bodyweight
EF	Ejection fraction
ESA	End-systolic area
ESA_{BW}	End-systolic area indexed to bodyweight
ESV	End-systolic volume
ESV_{BW}	End-systolic volume indexed to bodyweight
FAC	Fractional area change
PH	Pulmonary Hypertension
RV	Right ventricular
$RVDd^{base}$	Right ventricular diameter at end-diastole at the ventricular base / level of the tricuspid valve

$RVDd^{base}_{BW}$	Right ventricular diameter at end-diastole at the ventricular base / level of the tricuspid valve indexed to bodyweight
$RVDd^{mid}$	Right ventricular diameter at end-diastole at the level of the mid ventricle
$RVDd^{mid}_{BW}$	Right ventricular diameter at end-diastole at the level of the mid ventricle indexed to bodyweight
RVLd	Right ventricular length at end-diastole
$RVLd_{BW}$	Right ventricular length at end-diastole indexed to bodyweight
S’	Peak velocity of systolic tricuspid annular motion as determined by pulsed wave Doppler
SD	Standard deviation
SV	Stroke volume
SV_{BW}	Stroke volume indexed to bodyweight
TAPSE	Tricuspid annular plane systolic excursion
TEi	Myocardial performance index

b. Introduction

Myxomatous mitral valve disease (MMVD) is the most common acquired cardiac disease affecting dogs[1,2]. Evaluation of RV ventricular function is an important prognostic and decision-making tool in people with MMVD[3–6]. Owing to complex systolic and diastolic ventricular interdependence, dilation and dysfunction of the left ventricle can adversely affect RV function[7]. Pulmonary hypertension (PH) can also affect indices of RV function[8], is associated with poorer outcome[9], and can occur secondary to reactive vasoconstriction as a result elevated left atrial pressures in dogs with advanced MMVD[10].

Until recent years, assessment of the right heart has garnered relatively little attention in comparison to the left heart in both the human[4,11–14] and veterinary[15,16] literature. Given the asymmetric geometry of the right ventricle, lack of standardization of echocardiographic protocols has been a significant limitation on normal values for assessment of right ventricular (RV) structure and function[4,11,12,17,18]. However, the advent of newer technologies such as cardiac magnetic resonance imaging (cMRI) and real-time three-dimensional echocardiography (3DE) has led to an increased ability for accurate quantitative and qualitative assessment of the right ventricle[4,17]. Real-time 3DE has recently been used to evaluate RV function in anesthetized[17] dogs.

There is a relative paucity of data in the veterinary literature regarding RV function in dogs with acquired heart disease and, more specifically, MMVD. Right ventricular myocardial performance index (TEi) is independently associated with cardiac death in dogs with MMVD[19]. In a study of dogs with PH of various causes, including MMVD, dogs with severe PH were shown to have reduced TAPSE compared with control dogs[8].

Boxers with ARVC have also been demonstrated to have reduced RV ejection fraction when assessed by cMRI[20]. In dogs with congestive heart failure (CHF) due to mitral regurgitation, right heart chambers were shown to be enlarged and compressed when assessed by first pass radionuclide angiography[21]. In one study, peak velocity of systolic tricuspid annular motion as determined by pulsed wave Doppler (S') did not differ between dogs with varying degrees of MMVD and normal dogs[22]; however, another study showed both S' and TAPSE were associated with MMVD[23].

To the authors' knowledge there are no studies evaluating RV function using 3DE in dogs with MMVD. Our aims were to prospectively measure conventional (2D, m-mode, Doppler) echocardiographic and 3D echocardiographic indices of RV systolic function in dogs with various stages of MMVD, classified as per the guidelines of the American College of Veterinary Internal Medicine (ACVIM), and to compare the results to those from normal, healthy dogs. We hypothesized that indices of RV systolic function would be reduced in dogs with MMVD when compared with normal, healthy, controls and that the degree of systolic dysfunction would be correlated with the clinical stage of the disease.

c. Animals, Materials and Methods

Animals

Between June 2015 and August 2016, dogs recruited from staff and students of the Virginia-Maryland College of Veterinary Medicine as well as client-owned dogs presented to the cardiology service at Veterinary Teaching Hospital of the same

institution were prospectively enrolled. In order to be enrolled, dogs had to either be healthy or be affected by MMVD. A complete history was obtained for all dogs. All dogs underwent physical examination and comprehensive echocardiographic examination including 2D, M-mode, Doppler and 3D echocardiography. Thoracic radiography, 6-lead surface electrocardiogram, complete blood count, serum biochemical analysis and urinalysis were performed at the discretion of the attending clinician.

Dogs weighing < 5kg or > 25 kg, dogs with evidence of significant non-cardiac systemic disease, dogs that required sedation to facilitate echocardiography and dogs with echocardiographic evidence of congenital or acquired cardiac disease other than MMVD were all excluded. Boxers and English Bulldogs were excluded due to the risk of occult arrhythmogenic right ventricular cardiomyopathy in these breeds[24,25]. Dogs with echocardiographically confirmed MMVD and apparently healthy dogs with murmurs louder than grade II/VI were excluded from the control group. MMVD was diagnosed based on the presence of thickened and/or prolapsed mitral valve leaflets on 2D echocardiography accompanied by mitral regurgitation identified by color Doppler imaging. Dogs with MMVD were staged according to the guidelines of the American College of Veterinary Medicine[1]. Diagnosis of left-sided congestive heart failure was made in patients with a characteristic mitral regurgitant murmur that had compatible clinical signs and documented radiographic evidence of pulmonary edema. This study was approved by the Institutional Animal Care and Use Committee of Virginia Tech (IACUC # - 15-061).

Echocardiographic examination

Conventional Echocardiography

All echocardiographic studies were performed by a board-certified cardiologist (MB, JA, SL) or by a cardiology resident (MA) under the supervision of a board-certified cardiologist. All studies were performed using an Artida echocardiographic system[a] with simultaneous ECG recording. Transducer selection was based on patient size and preset for image optimization. Dogs were gently restrained in right and left lateral recumbency, and standard echocardiographic planes directed from right and left windows were obtained[26]. Datasets were digitally captured for later off-line analysis on a dedicated digital workstation utilizing a dedicated software package[b]. All measurements of 2D, m-mode and Doppler RV indices were performed by a single observer (MA) from left-apical 4-chamber views optimized for the right-heart. The following measurements were obtained: end-diastolic RV diameter at the ventricular base / level of the tricuspid valve ($RVDd^{base}$), end-diastolic RV diameter at the mid-ventricle ($RVDd^{mid}$), right ventricular end-diastolic length (RVLd), RV end-diastolic area (EDA), RV end-systolic area (ESA), RV fractional area change (FAC), tricuspid annular plane systolic excursion (TAPSE) and peak velocity of systolic tricuspid annular motion as determined by pulsed wave Doppler (S') as previously described[15,16,27]. An average of at least three, representative, usually consecutive measurements was recorded for each echocardiographic variable.

Real-time three-dimensional echocardiography

All dogs underwent 3DE examination, as a continuation of the initial echocardiogram. Details of this 3DE examination are described in section 2 of this thesis.

Briefly, real-time 3DE datasets were acquired using a full matrix array transducer with 3D functionality[c] directed from a left-apical acoustic window. Transducer alignment was guided by a multi-plane real-time view, comprising of two orthogonal long-axis views (corresponding to a standard left-apical four-chamber view and a standard left-apical two-chamber view) and three short-axis views. Elevation, depth, and azimuth were adjusted to ensure the entire right ventricle was included. 3DE datasets were obtained with the acquisition modality set to full 4D mode, using data from four consecutive heartbeats. A minimum of three consecutive datasets from each patient was stored for later off-line analysis.

Image analysis

The technique for analysis of 3DE RV datasets is described in section 2 of this thesis. To summarize, all 2D, M-mode, Doppler and 3D RV echocardiographic studies were analyzed off-line by a single observer (MA) using workstations equipped with a dedicated software package[d]. A semi-automated procedure was used to generate a model of the RV using imported 3DE datasets as previously described. All datasets were manually reviewed to ensure correct software tracking and to correct any tracking errors. A final 3D RV model was generated and the following measurements of 3D end-diastolic volume (EDV), 3D end-systolic volume (ESV), 3D stroke volume (SV) and 3D ejection fraction (EF) were automatically obtained.

Data analysis

The study population was divided into a control group and three MMVD groups based on the clinical stage of MMVD according to ACVIM guidelines[1] (i.e. stages B1, B2 and C). Two-dimensional and 3DE indices of RV function were then compared between all 4 groups. Based on scaling exponents derived from a concurrent study on datasets from the 22 healthy control dogs, 2D and 3D echocardiographic variables with a known significant relationship to body size were then indexed according to the formula: $indexed\ variable = \frac{variable}{BW^{exp}}$ where 'exp' is the scaling exponent for each particular variable. This resulted acquisition of the following variables: indexed $RVDd^{base}$ ($RVDd^{base}{}_{BW}$; exp = 0.482), indexed $RVDd^{mid}$ ($RVDd^{mid}{}_{BW}$; exp = 0.461), indexed RVLd ($RVLd_{BW}$; exp = 0.392), indexed RV EDA (EDA_{BW}; exp = 0.93), indexed RV ESA (ESA_{BW}; exp = 0.966), indexed RV EDV (EDV_{BW}; exp = 1.176), indexed RV ESV (ESV_{BW}; exp = 1.216) and indexed RV SV (SV_{BW}; exp = 1.116).

Data were analyzed using a commercially available software package[e]. Normality was visually assessed using normal quantile plots. Homoscedascity was assessed by means of Levene's test. Normally distributed data are reported as mean ± standard deviation (SD). Non-normally distributed data are reported as median (range: min-max). Non-parametric data were assessed using a Kruskal-Wallis test with subsequent post-hoc analysis using Dunn's method for multiple comparisons between groups. A P-value of <0.05 was considered significant.

d. Results

Seventy-eight dogs were eligible for inclusion in this study. There were 40 males and 38 females. Represented breeds included mixed breed (n = 25), Cavalier King Charles Spaniel (n = 9), Dachshund (n = 7), Miniature Schnauzer (n = 6), Brittany Spaniel (n = 4), Shih Tzu (n = 4), Staffordshire Terrier (n = 3), Whippet (n = 3), Beagle (n = 2), Jack Russell Terrier (n = 2) and one each of various other breeds. The control group consisted of 22 dogs. Fifty-six dogs were affected by MMVD: 23 were ACVIM stage B1, 20 were ACVIM stage B2 and 13 were ACVIM stage C. Population characteristics of these groups are presented in table 1. There was no difference in sex between groups. ACVIM stage B2 and stage C dogs had a lower bodyweight compared to controls. Bodyweight did not differ between dogs with varying stages of MMVD. Control dogs were younger when compared to all other groups. There was no difference in age between dogs in various stages of MMVD.

Table 1. Descriptive statistics of control group and MMVD groups by ACVIM stage

Variable	Control (n = 22)	Stage B1 (n = 23)	Stage B2 (n = 20)	Stage C (n = 13)
Age (years)	4.5 (0.9-11.4)	9.9 * (3.6-15.9)	10.18* (7.1-15.3)	12.2* (6.1-14.9)
Bodyweight (kg)	15.75 (6-25)	11 (5.6-24)	8.4* (5-22.3)	7.5* (5.7-16.2)
Sex (Male/Female)	10/12	12/11	12/8	6/7

* *$p \leq 0.001$ compared to control dogs*

Table 2 summarizes the results of measured and calculated 2D, m-mode and Doppler echocardiographic variables. TAPSE was lower in ACVIM stage B1 dogs when compared to controls ($p < 0.001$). S' was higher in ACVIM stage B2 dogs when

compared to controls ($p < 0.001$) and when compared to ACVIM stage B1 dogs ($p < 0.01$). When compared with controls, $RVDd^{base}_{BW}$ was higher in stage B2 ($p < 0.05$) and in stage C dogs ($p < 0.05$). FAC was higher in stage B2 dogs compared to control dogs ($p < 0.01$). $RVDd^{mid}_{BW}$, $RVLd_{BW}$, EDA_{BW}, and ESA_{BW} did not differ between groups.

Table 2. Measured and calculated two-dimensional, m-mode and Doppler echocardiographic indices of right ventricular function by group

Variable	Controls (n = 22)	Stage B1 (n = 23)	Stage B2 (n = 20)	Stage C (n = 13)
TAPSE (mm)	13.7 (10.2-15.6)	9.8*** (7-15.8)	12.7 (6.6-15.7)	11.7 (6.9-17.9)
S' (m/s)	0.11 (0.07-0.19)	0.10 (0.07-0.17)	0.15**,† (0.11-0.19)	0.13 (0.12-0.18)
$RVDd^{base}_{BW}$	4.28 (3.51-5.32)	4.42 (3.54-5.66)	4.82* (3.31-5.91)	4.85* (4.15-6.65)
$RVDd^{mid}_{BW}$	5.6 +/- 0.73	5.13 (3.42-6.49)	5.12 (3.86-7.09)	4.89 (3.88-6.42)
$RVLd_{BW}$	12.88 (10.3-13.81)	13.04 +/- 1.63	13.1 (10.01-16.81)	10.2 (9.93-17.71)
EDA_{BW}	0.53 (0.34-0.68)	0.48 (0.26-0.66)	0.49 (0.34-0.96)	0.40 (0.29-0.75)
ESA_{BW}	0.25 (0.13-0.37)	0.22 (0.12-0.35)	0.20 (0.12-0.39)	0.21 (0.13-0.31)
FAC (%)	46.65 (8.41-58.47)	47.91 (32.57-63.73)	55.92** (39.94-63.27)	51.38 (33.73-57.09)

TAPSE, tricuspid annular plane systolic excursion; S', peak velocity of systolic tricuspid annular motion as determined by pulsed wave Doppler; $RVDd^{base}_{BW}$, right ventricular diameter at end-diastole at the ventricular base / level of the tricuspid valve indexed to bodyweight; $RVDd^{mid}_{BW}$, right ventricular diameter at end-diastole at the level of the mid ventricle indexed to bodyweight; $RVLd_{BW}$, right ventricular length at end-diastole indexed to bodyweight; EDA_{BW}, right ventricular end-diastolic area indexed to bodyweight; ESA_{BW}, right ventricular end-systolic area indexed to bodyweight; FAC, fractional area change.

**p < 0.05 vs. controls.*

***p < 0.01 vs. controls.*

****p < 0.001 vs. controls.*

†p < 0.01 vs. stage B1.

Three-dimensional echocardiographic indices of RV function are summarized in table 3. RV EDV_{BW} was decreased in ACVIM stage B1 ($p < 0.05$), stage B2 ($p < 0.05$)

and stage C ($p < 0.01$) dogs when compared to controls. RV ESV_{BW} was lower dogs with stage B2 ($p < 0.01$) and stage C MMVD when compared to the control group. RV SV_{BW} was lower in ACVIM stage B2 dogs when compared to controls ($p < 0.05$). RV 3D EF was higher in ACVIM stage C dogs compared to controls ($p < 0.01$) and to ACVIM stage B1 dogs ($p < 0.05$).

Table 3. Measured and calculated three-dimensional echocardiographic indices of right ventricular function by group

Variable	Controls (n = 22)	Stage B1 (n = 23)	Stage B2 (n = 20)	Stage C (n = 13)
EDV_{BW}	0.68 (0.34-1.20)	0.46* (0.19-0.94)	0.46* (0.23-0.89)	0.40** (0.22-0.66)
ESV_{BW}	0.38 (0.20-0.67)	0.25 (0.07-0.47)	0.22** (0.11-0.45)	0.18*** (0.09-0.36)
SV_{BW}	0.31 (0.13-0.53)	0.19* (0.07-0.49)	0.26 (0.10-0.57)	0.27 (0.11-0.40)
EF (%)	37.65 ± 4.66	42.48 (21.4-61.21)	43.75 (31.67-63.74)	52.96**,† (26.15-66.79)

EDV_{BW}, right ventricular end-diastolic volume indexed to bodyweight; ESV_{BW}, right ventricular end-systolic volume indexed to bodyweight; SV_{BW}, right ventricular stroke volume indexed to bodyweight; EF, right ventricular three-dimensional ejection fraction.
**p < 0.05 vs. controls.*
***p < 0.01 vs. controls.*
****p < 0.001 vs. controls.*
†p < 0.05 vs. stage B1.
†††p < 0.01 vs. stage B1.

e. Discussion

This study demonstrates that multiple indices of RV function differ between dogs with varying severity of MMVD and normal healthy dogs. In particular, significant differences were identified for all 3DE indices investigated. The findings are somewhat unexpected. EDV and ESV both decrease with increasing severity of MMVD, while 3D EF was increased in dogs with left-sided congestive heart failure. Three-dimensional SV

was largely maintained but was increased in stage B1 dogs compared with controls. The decrease in EDV suggests there is progressively reduced RV filling with advancing severity of MMVD. It has been suggested that this could be due to the compression of the right ventricle secondary to increasing left ventricular dilation. Carlsson et al previously demonstrated that the right heart chambers become compressed with advanced MMVD when measured using first pass radionuclide angiography[21]. That same study measured total right heart size based on area and so, with the complex geometry of the right ventricle, is not possible to determine how this relates to RV volume. Another possible reason for these findings may be underfilling of the right heart as a result of decreased forward flow in patients with significant mitral regurgitation and/or left ventricular systolic dysfunction. It is also possible that the reduction in RV volumes observed, is the result of diuresis or other vasoactive therapies. Pimobendan and atenolol have been shown to respectively increase and decrease 2D, m-mode and Doppler indices of RV systolic function in healthy dogs[28].

In the present study, differences were also identified in several 2D, m-mode and Doppler RV function indices. We found that TAPSE was decreased in dogs with ACVIM stage B1; however, this effect was not identified in dogs with stage B2 or stage C disease. Olsen et al previously reported an association between TAPSE reduction and stage of MMVD in dogs[23]. Discordance with this finding in the present study may be attributable to small group size, in particular for dogs with stage C MMVD. In people, the prognostic implications of reduced TAPSE in patients with left-sided are unclear. One study of patients with heart failure with preserved ejection fraction showed that reduced TAPSE was predictive of hospitalization but not of their primary endpoint which was a composite

of cardiovascular hospitalization and death[29]. The prognostic implications of reduced TAPSE in dogs with MMVD is unknown, and further studies are required to elucidate this.

Peak velocity of systolic tricuspid annular motion was increased in ACVIM stage B2 dogs when compared to controls and to stage B1 dogs. The significance of this finding is unclear. There is disagreement in the literature regarding this variable in dogs with MMVD. Olsen et al demonstrated that S' was reduced in dogs with congestive heart failure due to MMVD[23]. That study also demonstrated that S' increased with age. However, another study by Baron Toaldo et al failed to find any significant association between S' and clinical stage of MMVD[22].

Another finding of our study was that $RVDd^{base}_{BW}$ increased in dogs with more severe MMVD. On first impressions, it would appear this result is discordant with our 3D volumetric results. However, linear 2DE dimensions can vary significantly depending on minor adjustments of transducer position and so may easily be under- or over-estimated[30,31]. Moreover, 3DE has been shown to be more reproducible and accurate than 2DE in the determination of RV dimensions[31,32]. This may also provide an explanation for the finding of increased FAC in dogs with stage B2 MMVD compared with controls.

Unsurprisingly, dogs with MMVD were older than control dogs in this study. This is not an unexpected finding, especially when considering the natural history of the disease[2]. We also found that dogs with more advanced MMVD (stage B2 and stage C) were smaller compared to controls. However, in order to mitigate the effect of size difference between groups we were able to index variables with known allometric

relationships to body size against bodyweight using exponents derived from a concurrent study in our control group.

Limitations

The present study has several limitations that should be addressed. Firstly, the small sample size may have limited our ability to detect small differences between groups. Nonetheless, the study size was sufficient to identify differences for several indices of RV function. Secondly, there was no standardization of therapy for patients with advanced stage B2 or stage C MMVD. It is unclear as to the degree of effect various medications would have had on these patients and it is possible that therapy could have contributed to the observed results. Another limitation is that 2D and 3DE datasets were obtained by multiple operators. The possibility exists that intra-operator variability in image acquisition could have influenced our results. Additionally, age- and weight-matching was not performed. Age-matching is not feasible given the natural history of MMVD. However, as previously discussed we were able to eliminate the effect of size differences between groups for the majority of variables by indexing them to bodyweight. Finally, longitudinal follow-up was not performed in our study population. It is unclear as to how various indices of RV systolic function may change over time as MMVD progresses and how outcome may be affected.

Conclusions

To conclude, 3D RV EDV and ESV are decreased and 3D RV EF is increased in dogs with advanced MMVD when compared to normal dogs. With regards conventional

echocardiographic indices of RV function, TAPSE was decreased in dogs with stage B1 MMVD, S' and FAC were increased in dogs with stage B2 MMVD and $RVDd^{base}_{BW}$ was increased in dogs with stage B2 and stage C MMVD. Further studies are necessary to determine the clinical and prognostic implications of these findings.

Footnotes

a. Artida, Toshiba Medical Systems, Tokyo, Japan
b. Image Arena, TomTec Imaging Systems, Unterschleissheim, Germany
c. PST-25SX matrix array transducer, Toshiba Medical Systems, Tokyo, Japan
d. 4D RV-Function, TomTec Imaging Systems, Unterschleissheim Germany
e. JMP Pro 11, SAS Institute Inc., Cary, N.C., USA

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3. Conclusions

Real-time 3DE is an evolving technology and in recent years has established itself as an important modality for the assessment of RV function in people. Despite this, there is little information on 3DE assessment of RV function in dogs. In the 1st study presented, acquisition and assessment of 3DE RV function indices are demonstrated to be feasible in conscious, healthy dogs with acceptable intra- and inter-observer variability. The study also shows that 3D volumetric indices are related to body size and proposes prediction intervals for 3DE-derived end-diastolic RV volume, end-systolic RV volume, and RV stroke volume.

In addition, assessment of RV function is a significant prognostic and decision-making tool in humans with MMVD. The 2nd study presented demonstrates that 3D EDV and 3D ESV are decreased in dogs with MMVD when compared to healthy controls and this decrease appears to be associated with the clinical severity of MMVD. It also shows that 3D EF is increased in dogs with CHF due to MMVD. In addition, the 2nd study also shows that TAPSE is reduced in dogs with stage B1 MMVD, that S' is increased in dogs with stage B2 MMVD and that the basal RV diameter is increased in dogs with stage B2 and stage C MMVD. Further studies are required to investigate the clinical and prognostic utility of these findings.

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