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Echocardiographic assessment of right heart size and function in dogs with pulmonary valve stenosis☆

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Abstract *Introduction/Objectives:* We sought to determine the prevalence and clinical significance of right heart remodeling and right ventricular (RV) dysfunction in dogs with pulmonary valve stenosis (PS). We also sought to evaluate repeatability of several measurements of severity of PS, right heart size, and RV function in dogs with PS.
Animals, materials and methods: Several indices of right atrial (RA) size and RV size and function were prospectively evaluated in 48 dogs with PS. Regression analysis was used to determine if indices of right heart size and function were independently associated with maximum transpulmonary pressure gradient (max PG) and adverse clinical findings (exercise intolerance, syncope, or right heart failure). Eight dogs underwent a second echocardiogram performed by the same operator to assess repeatability of the echocardiographic indices, which was quantified by coefficient of variation (CV) and repeatability coefficient.
Results: Increased RA size (81%), increased RV wall thickness (83%), and decreased tricuspid annular plane systolic excursion (TAPSE [81%]) were common. Right atrial size, end-diastolic RV area, and RV wall thickness were independently associated with max PG. Decreased TAPSE was independently associated with adverse clinical findings. All indices except RA area (18.6%) and RV systolic velocity (20.7%) had CVs <15%. Repeatability coefficients are available to help distinguish a true change versus measurement variability during serially obtained exams.

☆ This work was performed at the William R. Pritchard Veterinary Medical Teaching Hospital at the University of California, Davis, Davis, CA.

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Conclusions: Right heart remodeling and RV dysfunction are common in dogs with PS and are associated with echocardiographic and clinical severity. Results support the quantitative assessment of right heart size and function in dogs with PS.

Abbreviations

CV	coefficient of variation
iFAC	fractional area change indexed to body weight
PFO	patent foramen ovale
iPVA	pulmonary valve area indexed to body surface area
iRAA	right atrial area indexed to body weight
iRVAd	right ventricular area at end-diastole indexed to body weight
iRVIDd	right ventricular internal dimension at end-diastolic indexed to body weight
iRVFWd	right ventricular free wall at end-diastole indexed to body weight
iRV S'	peak systolic RV myocardial velocity at the lateral tricuspid annulus indexed to body weight
iTAPSE	tricuspid annular plane systolic excursion indexed to body weight
max PG	maximum transpulmonary pressure gradient
PS	pulmonary valve stenosis
PV	pulmonary valve
RA	right atrium/atrial
$RC_{95\%}$	95% repeatability coefficient
RV	right ventricle/ventricular
VTI_{AV}	velocity time integral of the aortic valve
VTI_{PV}	velocity time integral of the pulmonary valve
wSD	within-subject standard deviation

Introduction

Pulmonary valve stenosis (PS) is one of the most commonly diagnosed congenital heart diseases in dogs [1,2]. The majority of dogs present with an incidentally detected murmur but occasionally present with exercise intolerance, syncope, or right heart failure [3]. Outcome data from retrospective studies have suggested that clinical signs (exercise intolerance, syncope, or right heart failure), transpulmonary pressure gradient, age, pulmonary annular hypoplasia, and tricuspid regurgitation adversely affect survival of dogs with PS [3—5].

In human and veterinary medicine, there is an increased awareness of the clinical and prognostic importance of right ventricular (RV) size and function in a variety of cardiovascular diseases [6—8]. The RV appears to perform and adapt differently depending on the type and timing of hemodynamic stressors. In contrast to the adult myocardium, the fetal myocardium and the neonatal myocardium exhibit a robust tolerance to increased afterload through cardiomyocyte hyperplasia and the ability to increase the number of capillaries [9]. Intriguing studies in children have demonstrated that RV performance is more dramatically affected by idiopathic pulmonary arterial hypertension compared to isolated PS [10—12]. However, children with PS are still considered at risk for eventual RV dilation and failure, and early intervention is strongly advised to preserve RV function, reduce risk of clinical signs, and improve survival [8,9,13].

Little is known about the prevalence and clinical significance of right heart remodeling and RV dysfunction in dogs with PS. Therefore, we sought to determine the prevalence of right atrial (RA) and RV hypertrophy and RV systolic dysfunction in dogs with PS. We also sought to determine if RA and RV hypertrophy and RV systolic dysfunction are associated with the severity of PS or adverse clinical findings. Given there are few studies detailing echocardiographic measurements of the right heart in dogs with PS, a secondary objective was to evaluate the repeatability of several measurements of PS severity, right heart size, and RV function in dogs with PS. We hypothesized that RA and RV hypertrophy and RV systolic dysfunction are common and that right heart remodeling and RV systolic dysfunction are associated with echocardiographic (maximum pressure gradient) and clinical severity (clinical signs or right heart failure) of PS.

Animals, Materials, and Methods

Animals

This was a prospective observational study that utilized a convenience sample of dogs that were already scheduled to undergo an echocardiographic examination as part of their visit to our hospital. This echocardiographic examination did not deviate from standard practice at our hospital. Some dogs were referred for the purpose of an alternate study [14]. Institutional Animal Care and Use Committee approval (protocol #: 19199) and owner consent were obtained for the eight dogs that underwent an additional echocardiographic examination for the repeatability studies. Dogs were consecutively enrolled over a 3.5-year period, provided they were diagnosed with PS and had a maximum pressure gradient (max PG) at the level of the pulmonary valve (PV) $>$40 mmHg. Dogs were excluded from the study if they were taking medications known to affect the cardiovascular system (including sedatives) prior to or at the time of the echocardiographic examination. Dogs were also excluded if they had any additional cardiovascular disease, systemic disease (based on history or physical exam), or a sustained pathological arrhythmia. The tricuspid valve apparatus was carefully evaluated with echocardiography for evidence of tricuspid valve dysplasia (e.g., overt irregularities, thickening, or both of the tricuspid valve or chordae tendineae). Dogs were excluded if they were thought to be affected by tricuspid valve dysplasia. Dogs diagnosed with a patent foramen ovale (PFO) based on an agitated saline contrast study were not excluded provided the shunting was considered mild and their hematocrit was $<$55%. Dogs with trivial or mild regurgitation of the tricuspid, mitral, and aortic valves (assessed subjectively) were not excluded, provided the valve appearance was considered normal. Owner reported exercise intolerance and syncope were recorded. Right heart failure was defined as ultrasonic evidence of ascites in addition to a subjectively dilated caudal vena cava.

Study design

All dogs underwent an echocardiographic examination (see below), an agitated saline contrast study to screen for a PFO, and, if present, hematocrit assessment as part of routine practice at our hospital. Eight dogs were selected based on owner availability and consent to undergo an additional echocardiographic examination performed by the same operator 2—4 h after the first one for echocardiographic measurements repeatability studies. Recommendations for pulmonary balloon valvuloplasty and to assess for an anomalous coronary artery with computed tomographic angiography or fluoroscopic angiography were made at the discretion of the attending clinician.

Echocardiographic examination

Image acquisition

All echocardiographic studies[a] were performed by a board-certified veterinary cardiologist or a cardiology resident under the supervision of a board-certified veterinary cardiologist. Sonographers used a standardized echocardiographic imaging protocol and standard imaging planes [15] for each dog. The right parasternal long axis five-chamber view optimized for the left ventricular outflow tract and ascending aorta was used to measure the aortic valve diameter, often with the aid of the zoom feature. A right parasternal short axis basilar view optimized for the RV outflow tract was used to acquire the maximum transpulmonary velocity using continuous-wave Doppler using two-dimensional and color Doppler guidance. The subcostal view was used to acquire pulsed-wave Doppler profiles at the level of the aortic valve hinge points. A left apical four-chamber view optimized for the right heart was used to acquire M-mode recordings of tricuspid annular plane systolic excursion (TAPSE), right heart dimensions, and pulsed-wave tissue Doppler-derived peak systolic RV myocardial velocity at the lateral tricuspid annulus (RV S') as previously described [16—18]. Optimization for the right heart typically involved transducer placement one intercostal space cranial to the standard left apical four-chamber view with varying degrees of caudal angulation. A concerted effort was made to avoid foreshortening of the right heart. Attempts were made to maintain visualization of both atria and of both ventricles and their respective apexes. Visualization of the left ventricular outflow tract was avoided. For all spectral or tissue Doppler recordings, the baseline, scale, and gain were manually adjusted in an attempt to optimize the Doppler signal.

[a] Philips EPIQ 7C or iE33, Philips Healthcare, Andover, MA, USA.

Echocardiographic measurements and calculations

All echocardiographic measurements were performed by a single investigator (LCV) at an off-cart workstation[b]. Measurements were performed in the same order for each dog, with measurements of PS severity (i.e., Doppler evaluations of the pulmonary valve) performed last. Values for each echocardiographic variable consisted of the average of three usually consecutive measurements. The heart rate recorded represented the mean of three instantaneous heart rates determined from the M-mode tracing acquired for the TAPSE assessment. The aortic valve diameter (in centimeters) was measured in early to mid-systole between the hinge points of the maximally opened aortic valve cusps. The pulsed-wave Doppler-derived profile of the aortic valve was determined by tracing the outer edge of the modal velocities (denser signals) throughout systole [19]. The software package calculated the velocity time integral (VTI) of the aortic valve (VTI_{AV}). The same technique was applied to the continuous-wave Doppler profile of the PV to determine VTI of the pulmonary valve (VTI_{PV}). Modal velocities were traced and fine linear signals at the peak of the curve were avoided [19]. Maximum transpulmonary velocity was measured at the peak of the outer edge of the denser signal while avoiding measurement of fine linear signals [19]. Maximum velocity was converted to a max PG using the simplified Bernoulli equation: pressure gradient $= 4 \times$ velocity $(m/s)^2$. Maximum right atrial area indexed to body weight (iRAA) was measured by manually tracing the internal border of the RA at ventricular end-systole [18]. A straight line drawn from hinge point to hinge point across the tricuspid valve annulus (atrial side) defined the boundary of the RA and RV. The thickness of the right ventricular free wall at end-diastole indexed to body weight (iRVFWd) and right ventricular internal dimension at end-diastole indexed to body weight (iRVIDd) in the minor axis were both measured at the mid-ventricular level as previously described [18]. RV areas at end-diastole and end-systole were determined using planimetry. A straight line drawn from hinge point to hinge point (ventricular side) across the tricuspid valve annulus was used to define the boundary between the RV and RA for these measurements.

Velocity time integral ratio (VTI_{AV}/VTI_{PV}) was calculated as VTI_{AV} (cm) $\div$ VTI_{PV} (cm). Cross-sectional area of the aortic valve was calculated as: $\pi \times$ (aortic valve diameter $\div$ 2)^2. The continuity equation was used to estimate the effective orifice area of the PV or pulmonary valve area (PVA) as follows: PVA (cm^2) = (cross-sectional area of the aortic valve $\times$ VTI_{AV}) $\div$ VTI_{PV} [14,20]. The PVA was then indexed to each dog's body surface area (iPVA) as follows: PVA $\div$ body surface area (m^2). Indices of right heart size (iRAA, iRVIDd, iRVAd, and iRVFWd) were indexed (i) to body weight (in kg) using previously published scaling exponents: iRAA = $cm^2/kg^{0.71}$, iRVIDd = $cm/kg^{0.33}$, iRVAd = $cm^2/kg^{0.62}$, and iRVFWd = $cm/kg^{0.25}$ [18]. Right ventricular fractional area change (FAC) was calculated as ([RV area at end-diastole $-$ RV area at end-systole] $\div$ RV area at end-systole) $\times$ 100. Indices of RV systolic function (TAPSE, FAC, and RV S') were indexed (i) to body weight (kg) using previously published scaling exponents: iTAPSE in $mm/kg^{0.297}$, iFAC in $\%/kg^{-0.097}$, and iRV S' in $cm/sec/kg^{0.23}$) [17].

Echocardiographic repeatability

Intra-operator within-day repeatability was determined by having a single investigator (LCV) measure the two echocardiographic examinations from the eight dogs selected to assess within-day repeatability. The same operator performed the first and repeat echocardiographic examination but this was not necessarily by the same investigator that performed the measurements. Measurements were performed at least one month before with the investigator blinded to previous measurements.

Statistical analyses

Statistical analyses were performed using a commercial computer software package[c]. Descriptive statistics were generated and the Shapiro—Wilk test was used for normality testing for all continuous data. Continuous data were reported as mean (standard deviation) if normally distributed and median (interquartile range) if normality testing failed. Categorical data were summarized as frequencies and proportions. Differences of continuous data comparing dogs with max PG $\leq$ 80 mmHg versus dogs with max PG $>$ 80 mmHg were evaluated by a Mann—Whitney test. Differences in proportions comparing dogs with max PG $\leq$ 80 mmHg versus

[b] Syngo® Dynamic Workplace, Siemens Medical Solutions, Inc., Malvern, PA, USA.

[c] MedCalc Statistical Software for Windows 10, Version 18.11.3, MedCalc Software bvba, Ostend, Belgium.

Table 1 Clinical data from 48 dogs with PS.

Clinical variables	All dogs (n = 48)	Max PG ≤ 80 mmHg (n = 11)	Max PG > 80 mmHg (n = 37)
Body weight (kg)	16.9 (10.0)	15.1 (11.2)	17.4 (9.7)
Age (years)	1.1 (0.4–2.6)	0.6 (0.2–1.7)	1.4 (0.5–3.2)
Female: number (%)	21 (44%)	1 (9%)	20 (54%)
PFO: number (%)[a]	8 (17%)	0 (0%)	8 (22%)
Anomalous CA: number (%)[b]	7 (18%)	1 (9%)	6 (16%)
Clinical signs: number (%)	17 (35%)	2 (18%)	15 (41%)
Exercise intolerance: number (%)	8 (17%)	0 (0%)	8 (22%)
Syncope: number (%)	5 (10%)	0 (0%)	5 (14%)
Right heart failure: number (%)	5 (10%)	2 (18%)	3 (8%)

CA: coronary artery; Max PG: maximum transpulmonary pressure gradient; PFO: patent foramen ovale.
[a] Not evaluated in four dogs.
[b] Not evaluated in 10 dogs.

dogs with max PG > 80 mmHg were evaluated using a Fisher's exact test. Simple (univariable) linear regression analyses were performed to initially determine the strength of association of selected echocardiographic indices to the echocardiographic severity of PS as defined by max PG. Variables that yielded a $p<0.2$ based on the univariable linear regression analyses were entered into a multiple linear regression model in a backward stepwise manner. Similarly, univariable logistic regression analyses were performed to determine the strength of association of selected clinical and echocardiographic indices to the clinical severity of PS as defined by the presence of clinical signs (syncope or exercise intolerance) or right heart failure. Variables that yielded a $p<0.2$ based on univariable logistic regression analyses were entered into a multiple logistic regression model in a backward stepwise manner. A post-hoc receiver operating characteristic analysis was performed to determine a clinically relevant cutoff for iTAPSE for predicting risk of adverse clinical findings of exercise intolerance, syncope, or right heart failure. The clinically relevant cutoff value was chosen based on the highest Youden index.

Within-day repeatability was quantified by the within-subject coefficient of variation (CV) and repeatability coefficient ($RC_{95\%}$), which were both determined from a one-way ANOVA using individual dog as the grouping variable. The within-subject standard deviation (wSD) was determined using the square root of within-subject variance (residual mean square). The CV was calculated as: (wSD ÷ population mean) × 100 [21], and the $RC_{95\%}$ was calculated as: $1.96 \times \sqrt{2} \times$ wSD [22]. The $RC_{95\%}$ provides an estimate (with 95% confidence interval) of the absolute difference (in the same units as the measurement under consideration) between two repeated measurements, assuming there is no true change in the measured variable [22]. In other words, if the absolute difference of repeated measurements is beyond the $RC_{95\%}$, a true (clinically significant) change in the measured variable is very likely. A probability of $p<0.05$ was considered statistically significant.

Results

Clinical data from the 48 dogs enrolled in this study are summarized in Table 1. Breeds represented were mixed breeds (n = 14), pit bull terriers (n = 10), Bulldogs (n = 8), French bulldogs (n = 6), and one of each of the following breeds: American cocker spaniel, Entlebucher mountain dog, German shepherd dog, Basset hound, Blood hound, Manchester terrier, Redbone coonhound, Chihuahua, Collie, and an Anatolian shepherd. Eight dogs (17%) were diagnosed with a PFO that exhibited some right-to-left shunting, and their packed cell volumes ranged from 35 to 54%. Seven dogs were diagnosed with an anomalous prepulmonic coronary artery (six Bulldogs and one French bulldog). Seventeen of the 48 dogs (35%) exhibited exercise intolerance, syncope, or right heart failure. The majority of these (15 dogs) had a max PG > 80 mmHg. Sixteen dogs exhibited one clinical sign (five exhibited right heart failure, 4 experienced syncope, and 7 had exercise intolerance) and one dog exhibited exercise intolerance and syncope. Exercise intolerance or syncope was not reported in any of the dogs with right heart failure.

A summary of the echocardiographic data is presented in Table 2. Eleven of 48 dogs (23%) had a max PG ≤ 80 mmHg and 37 of 48 (77%) had a max PG > 80 mmHg. Eighty-one percent (39 of 48) of dogs had RA enlargement when assessed by iRAA and the majority of these (33 dogs) had a max PG > 80 mmHg. Right atrial size (iRAA) was significantly ($p=0.02$) increased in dogs with max

Table 2 Echocardiographic data from 48 dogs with PS.

Echocardiographic variables	All dogs (n = 48)	Max PG ≤ 80 mmHg (n = 11)	Max PG > 80 mmHg (n = 37)	*p*-value
Max PG (mmHg)	115.1 (47.7)	59.0 (15.1)	131.8 (40.7)	n/a
iPVA (cm^2/m^2)	0.40 (0.17)	0.57 (0.14)	0.35 (0.14)	**<0.0001**
VTI_{AV}/VTI_{PV}	0.18 (0.06)	0.24 (0.04)	0.16 (0.05)	**<0.0001**
iRAA ($cm^2/kg^{0.71}$)	0.96 (0.79–1.22)	0.83 (0.56–0.96)	0.99 (0.82–1.33)	**0.02**
iRAA >0.76[a]: number (%)	39 (81%)	6 (55%)	33 (89%)	**0.02**
iRVIDd ($cm/kg^{0.33}$)	0.80 (0.14)	0.75 (0.11)	0.81 (0.15)	0.34
iRVIDd >0.94[a]: number (%)	7 (14%)	0 (0%)	7 (19%)	0.18
iRVAd ($cm^2/kg^{0.62}$)	0.89 (0.79–1.10)	0.84 (0.80–1.03)	0.90 (0.77–1.08)	0.70
iRVAd >1.33[a]: number (%)	2 (4%)	0 (0%)	2 (5%)	>0.999
iRVFWd ($cm/kg^{0.25}$)	0.52 (0.44–0.72)	0.37 (0.32–0.48)	0.60 (0.49–0.77)	**<0.0001**
iRVFWd >0.39[a]: number (%)	40 (83%)	4 (36%)	36 (97%)	**<0.0001**
iTAPSE ($mm/kg^{0.297}$)	4.0 (0.9)	4.7 (0.6)	3.7 (0.90)	**0.0001**
iTAPSE <4.8[b]: number (%)	39 (81%)	7 (64%)	32 (86%)	0.18
iFAC ($\%/kg^{-0.097}$)	61.6 (15.9)	65.1 (10.6)	60.6 (17.1)	0.35
iFAC <46.3%[b]: number (%)	8 (17%)	0 (0%)	8 (22%)	0.17
iRV S' ($cm/sec/kg^{0.233}$)	4.1 (3.2–5.4)	4.0 (3.6–6.5)	4.1 (3.0–5.1)	0.52
iRV S' <4.3[b]: number (%)	23 (48%)	6 (55%)	17 (46%)	0.74

AV: aortic valve; iFAC: fractional area change indexed to body weight; n/a: not applicable; iPVA: pulmonary valve area indexed to body surface area; iRAA: right atrial area indexed to body weight; iRVAd: right ventricular area at end-diastole indexed to body weight; iRVFWd: right ventricular free wall thickness at end-diastole indexed to body weight; iRVIDd: right ventricular interval dimension at end-diastole indexed to body weight; iRV S': right ventricular systolic myocardial velocity at the lateral tricuspid annulus indexed to body weight; iTAPSE: tricuspid annular plane systolic excursion indexed to body weight; Max PG: maximum transpulmonary pressure gradient; PV: pulmonary valve; VTI: velocity time integral.
P-value represents the comparison of Max PG ≤ 80 mmHg to Max PG > 80 mmHg.
Continuous data reported as mean (standard deviation) if normally distributed and median (interquartile range) if non-normality distributed.
Bolded values denote statistical significance.
[a] Upper 95% prediction interval [18].
[b] Lower 95% prediction interval [17].

PG > 80 mmHg (0.99 [0.82–1.33] $cm^2/kg^{0.71}$) compared to dogs with a max PG ≤ 80 mmHg (0.83 [0.56–0.96] $cm^2/kg^{0.71}$). Increased RV chamber size (assessed by iRVIDd and iRVAd) was uncommon (14% for iRVIDd and 4% for iRVAd). There were no significant differences ($p \geq 0.18$) in measurements of RV chamber size (iRVIDd and iRVAd) or the percent of dogs with RV enlargement when comparing dogs with a max PG ≤ 80 mmHg to dogs with a max PG > 80 mmHg. Increased thickness of the RV free wall (iRVFWd) was common (83%; 40 of 48 dogs), particularly in dogs with max PG > 80 mmHg (36 of 37 dogs). Right ventricular wall thickness (iRVFWd) was significantly ($p<0.0001$) increased in

Table 3 Results of the linear regression analyses to identify echocardiographic indices independently associated with the severity of PS (defined by maximum transpulmonary pressure gradient).

Variables	Univariable linear regression		Multiple linear regression
	R^2	*p*-value	*p*-value
iRAA	0.38	**<0.0001**	**0.03**
iRVIDd	0.10	**0.03**	0.11
iRVAd	0.07	**0.08**	**0.03**
iRVFWd	0.49	**<0.0001**	**0.02**
iTAPSE	0.27	**0.0002**	0.33
iFAC	0.04	**0.17**	–
iRV S'	0.15	**0.01**	–

iFAC: fractional area change indexed to body weight; iRAA: right atrial area indexed to body weight; iRVAd: right ventricular area at end-diastole indexed to body weight; iRVFWd: right ventricular free wall thickness at end-diastole indexed to body weight; iRVIDd: right ventricular interval dimension at end-diastole indexed to body weight; iRV S': right ventricular systolic myocardial velocity at the lateral tricuspid annulus indexed to body weight; iTAPSE: tricuspid annular plane systolic excursion indexed to body weight; R^2: coefficient of determination.
Bolded values denote statistical significance.

Table 4 Results of the logistic regression analyses to identify clinical or echocardiographic indices independently associated with the clinical severity of PS (defined by clinical signs or right heart failure).

Variables	Univariable logistic regression		Multiple logistic regression
	OR (95% CI)	*p*-value	*p*-value
Body weight	1.0 (0.97–1.10)	0.31	
Age	0.93 (0.64–1.36)	0.70	
Sex (male/female)	1.7 (0.51–5.82)	0.38	
Heart rate	1.0 (0.98–1.03)	0.62	
Max PG	1.02 (1.00–1.03)	**0.01**	–
iPVA	0.006 (0.0001–0.62)	**0.03**	0.48
VTI_{AV}/VTI_{PV}	0.0000 (0.0–4.1)	0.08	0.21
iRAA	8.3 (1.4–50.1)	**0.01**	0.30
iRVIDd	1.40 (0.02–102)	0.88	
iRVAd	0.24 (0.01–5.1)	0.34	
iRVFWd	80.9 (2.0–3254)	**0.01**	–
iTAPSE	0.26 (0.01–0.67)	**0.0008**	**0.02**
iFAC	0.04 (0.0006–2.53)	0.11	–
iRV S'	0.57 (0.33–0.98)	**0.02**	0.27

AV: aortic valve; CI: confidence interval; iFAC: fractional area change indexed to body weight; iPVA: pulmonary valve area indexed to body surface area; iRAA: right atrial area indexed to body weight; iRVAd: right ventricular area at end-diastole indexed to body weight; iRVFWd: right ventricular free wall thickness at end-diastole indexed to body weight; iRVIDd: right ventricular interval dimension at end-diastole indexed to body weight;; iRV S': right ventricular systolic myocardial velocity at the lateral tricuspid annulus indexed to body weight; iTAPSE: tricuspid annular plane systolic excursion indexed to body weight; Max PG: maximum transpulmonary pressure gradient; OR: odds ratio PV: pulmonary valve; VTI: velocity time integral.
Bolded values denote statistical significance.

dogs with max PG $>$ 80 mmHg (0.60 [0.49–0.77] $cm/kg^{0.25}$) compared to dogs with a max PG $\leq$ 80 mmHg (0.37 [0.32–0.48] $cm/kg^{0.25}$). Decreased TAPSE, an index of RV systolic function, was noted in 39 dogs (81%), 7 of which had a max PG $\leq$ 80 mmHg. TAPSE was significantly (p=0.0001) decreased in dogs with max PG $>$ 80 mmHg (3.7 [0.90] $mm/kg^{0.297}$) compared to dogs with a max PG $\leq$ 80 mmHg (4.7 [0.6] $mm/kg^{0.297}$).

Associations of the right heart echocardiographic indices to the severity of PS defined by max PG and clinical signs/right heart failure are presented in Tables 3 and 4, respectively. Increased RA size (iRAA), RV chamber size (iRVAd), and RV wall thickness (iRVFWd) each demonstrated a significant ($p \leq 0.03$) independent association with max PG based on multiple linear regression analysis. Only RV systolic function quantified by

Table 5 Intra-operator within-day repeatability of selected echocardiographic variables from eight dogs with PS.

Echocardiographic variables	Population mean	wSD	CV (%)	$RC_{95\%}$
PV Vmax (m/s)	4.92	0.31	6.4	0.87
Max PG (mmHg)	102.2	12.2	12.0	33.9
iPVA (cm^2/m^2)	0.41	0.05	12.1	0.14
VTI_{AV}/VTI_{PV}	0.18	0.02	9.9	0.05
iRAA ($cm^2/kg^{0.71}$)	1.11	0.21	18.6	0.57
iRVIDd ($cm/kg^{0.33}$)	0.85	0.09	10.3	0.24
iRVAd ($cm^2/kg^{0.62}$)	1.08	0.13	12.2	0.37
iRVFWd ($cm/kg^{0.25}$)	0.61	0.08	13.1	0.22
iTAPSE ($mm/kg^{0.297}$)	4.85	0.61	12.7	1.70
iFAC ($\%/kg^{-0.097}$)	56.2	5.79	10.3	16.0
iRV S' ($cm/sec/kg^{0.233}$)	5.23	1.08	20.7	3.0

AV: aortic valve; CI: confidence interval; CV: coefficient of variation; iFAC: fractional area change indexed to body weight; iPVA: pulmonary valve area indexed to body surface area; iRAA: right atrial area indexed to body weight; iRVAd: right ventricular area at end-diastole indexed to body weight; iRVFWd: right ventricular free wall thickness at end-diastole indexed to body weight; iRVIDd: right ventricular interval dimension at end-diastole indexed to body weight; iRV S': right ventricular systolic myocardial velocity at the lateral tricuspid annulus indexed to body weight; iTAPSE: tricuspid annular plane systolic excursion indexed to body weight; Max PG: maximum transpulmonary pressure gradient; PV: pulmonary valve; $RC_{95\%}$: 95% repeatability coefficient; VTI: velocity time integral; wSD: within-subject standard deviation.

iTAPSE demonstrated a significant (p=0.02) independent association with clinical signs (exercise intolerance or syncope) or right heart failure based on multiple logistic regression analysis (Table 4). The optimal cutoff of iTAPSE for the prediction of exercise intolerance, syncope, or right heart failure was $\leq$3.28 mm/kg$^{0.297}$ where sensitivity was 64.7% and specificity was 100% (area under the curve, 95% confidence interval = 0.77, 0.62–0.88; p=0.002; Youden index = 0.65).

CVs and $RC_{95\%}$ for intra-operator within-day repeatability are reported in Table 5. The CVs for PV Vmax and VTI_{AV}/VTI_{PV} were <10%. The majority of indices had CVs between 10% and 15% (max PG, iPVA, iRVIDd, iRVAd, iRVFWd, iTAPSE, and iFAC). The CVs for iRAA and iRV S' were highest at 18.6% and 20.7%, respectively.

Discussion

This investigation represents one of the first studies to prospectively evaluate quantitative measurements of right heart size and function in untreated dogs with PS. As hypothesized, our results show that increased RA size (iRAA), RV hypertrophy (increased iRVFWd), and RV systolic dysfunction (assessed by iTAPSE) were common in dogs with PS when compared to published reference intervals [17,18]. We found that iRAA, iRVAd, and iRVFWd were independently associated with the severity of PS (defined by max PG). However, only decreased iTAPSE was independently associated with the adverse clinical findings of exercise intolerance, syncope, or right heart failure in dogs with PS. We also found that most echocardiographic measurements of right heart size and function (all but iRAA and iRV S') exhibited intra-operator, within-day CVs <15%. This study also provides repeatability coefficients to help clinicians determine if true changes in echocardiographic variables are present during serial evaluations (when performed by the same evaluator) of dogs with PS thus suggesting regression or progression of disease. For example, our results suggest that to document a true decrease (i.e., beyond measurement variability) in PV Vmax or max PG on a serially obtained measurement when performed by the same sonographer, e.g., post-balloon valvuloplasty, one should see a decrease of at least 0.87 m/s or 33.9 mmHg, respectively.

PS causes an increase in RV systolic pressure, and RV hypertrophy subsequently develops to attempt to normalize wall stress and maintain stroke volume. Hypertrophy can lead to impaired filling and increased RA pressure. Increases in RA pressure might be exacerbated by tricuspid regurgitation that is often noted in patients with PS, and the RV might eventually dilate and fail [9]. Some or all of these changes may manifest clinically as exercise intolerance, syncope, or right heart failure (ascites ± pleural effusion). Given the pathophysiology of PS, the high prevalence of increased RA size (iRAA), RV hypertrophy (iRVFWd), and RV systolic dysfunction (decreased iTAPSE) were not unexpected. Similarly, it was not surprising to see that RA size, RV dilation, and RV hypertrophy were independently associated with a conventional index of PS severity (max PG). However, it was somewhat surprising that only decreased iTAPSE was independently associated with the clinical severity of PS.

TAPSE is one of the most widely used indices of RV systolic performance in dogs and humans [6]. Its simplicity and ease to acquire make it appealing for routine clinical use. Decreased TAPSE has demonstrated clinical and prognostic value in a variety of cardiovascular disease in dogs [23], cats [24–26], and humans [27–29]. Similar to humans [13], decreased TAPSE has been documented in dogs with PS but it improves in post-balloon valvuloplasty [14]. The clinical and prognostic significance of RV dysfunction has not been well studied in dogs or humans with isolated PS. However, studies of humans with tetralogy of Fallot or PS have suggested that TAPSE is useful for risk stratification and prognosis [30–32].

Previous studies have suggested that clinical signs (exercise intolerance, syncope, or right heart failure), increased max PG, age at diagnosis, pulmonary annular hypoplasia, and tricuspid regurgitation adversely affect outcome in dogs with PS [3–5]. However, these studies did not systemically evaluate right heart size and function. Given we found that indices of right heart size (iRAA, iRVAd, and iRVFWd) and function (iTAPSE) were independently associated with max PG and clinical signs, respectively, our results are suggestive of a link between these indices of right heart size and function and outcome. However, we believe these results should not be overinterpreted and warrant further study of the potential link between these echocardiographic indices and prognosis in dogs with PS. The association of decreased iTAPSE with adverse clinical findings is clinically relevant and suggests that RV dysfunction (defined by decreased iTAPSE) is associated with the clinical severity of PS. Decreased iTAPSE, and particularly values $\leq$ 3.28 mm/kg$^{0.297}$, might be indicative of clinical signs or right heart failure and might

impact monitoring or therapeutic recommendations.

Two of the five dogs affected with right heart failure had a max PG $\leq$ 80 mmHg. Both of these dogs had overt RV systolic dysfunction. This highlights a limitation of using solely max PG to define the severity of PS and confounds the analysis of comparing right heart size and function to max PG. Max PG is a flow-dependent index of PS severity that might not be reliable in states of altered RV function [14].

We believe that a noteworthy limitation of this study includes its cross-sectional design. Serial evaluations would have been ideal to help clarify the relationship between RV dysfunction and disease progression and risk for the development of clinical signs and right heart failure in dogs with PS. Unfortunately, longitudinal studies are challenging, time-consuming, and expensive. Natural history data of dogs with PS derived from prospective studies are undoubtedly needed. We also acknowledge that owner-reported exercise intolerance is subjective, non-standardized, and subject to the varying degrees with which owners are able to monitor and exercise their dogs. Similarly, episodes of syncope could have been missed or misjudged by owners. It would have been ideal to assess exercise capacity in a more objective and standardized manner with, for example, a 6-min walk test as has been reported in previous studies [33]. Exercise intolerance and syncope are clinical signs that are not specific to dogs with PS, and a thorough diagnostic evaluation was not performed in each dog to rule out other causes of these clinical signs. This represents a limitation, particularly given the number of brachycephalic breeds enrolled in our study. We acknowledge that standardization of echocardiographic image acquisition, particularly of the right heart and with multiple sonographers, is challenging. Thus, our results are subject to the variability associated with multiple sonographers and the difficulty of standardizing the imaging acquisition planes for the right heart.

Conclusions

Our results support the quantitative assessment of right heart size and function in dogs with PS. Indices of right heart size (iRAA, iRVAd, and iRVFWd) and function (iTAPSE) were associated with the clinically and prognostically important findings of clinical signs and max PG in dogs with PS. Dogs with reduced iTAPSE, especially those with values $\leq$3.28 mm/kg$^{0.297}$, might be at risk for the adverse clinical findings of exercise intolerance, syncope, or right heart failure. Our results support the need for further investigations of the clinical and prognostic significance of right heart size and function in dogs with PS.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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