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The prevalence of pulmonary hypertension in Cavalier King Charles spaniels compared with other breeds with myxomatous mitral valve disease☆

S. Sudunagunta, BVetMed[a,*], D. Green, BVSc[b], R. Christley, BVSc, PhD[c], J. Dukes-McEwan, PhD[a]

[a] *Cardiology Service, Small Animal Teaching Hospital, Institute of Veterinary Science, University of Liverpool, Neston, CH64 7TE, UK*
[b] *Waikanae Beach, Wellington, 5036, New Zealand*
[c] *Department of Epidemiology and Population Health, Institute of Infection and Global Health, University of Liverpool, Neston, CH64 7TE, UK*

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KEYWORDS
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Abstract *Introduction:* Pulmonary hypertension (PH) is a common consequence of myxomatous mitral valve disease (MMVD). Cavalier King Charles spaniels (CKCS) are frequently affected with MMVD and appear to have different disease progression compared to other dogs. This study aimed to determine if CKCS are more likely to develop PH as a result of MMVD than dogs of other breeds. A secondary aim was to explore whether breed or PH impacted survival.

Abbreviations: ACVIM, American College of Veterinary Internal Medicine; AF, atrial fibrillation; CKCS, Cavalier King Charles spaniel; E vel, transmitral E wave velocity; E/IVRT, ratio of E wave velocity to isovolumic relaxation time; ESVI, end-systolic volume index; LA, left atrium; LA/Ao, left atrium-to-aortic ratio; L-CHF, left-sided congestive heart failure; LVIDDn, left ventricular internal diameter in diastole, indexed for body weight; LVIDSn, left ventricular internal diameter in systole indexed for body weight; MMVD, myxomatous mitral valve disease; PH, pulmonary hypertension; PR, pulmonic regurgitation; ReCHF, right-sided congestive heart failure; TR, tricuspid regurgitation.

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* Corresponding author.
E-mail address: ssudun@liv.ac.uk (S. Sudunagunta).

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Degenerative valvular disease

Animals: A total of 187 dogs diagnosed with MMVD, 94 CKCS and 93 non-CKCS, were included in this study.
Methods: This is a retrospective review of dogs with MMVD. Data were analyzed for presence of PH, congestive heart failure (CHF) and echocardiographic variables including the ratio between mitral E wave velocity (E vel) and isovolumic relaxation time (E/IVRT) and were compared between CKCS/non-CKCS and dogs with/without PH. Survival analysis was also performed.
Results: American College of Veterinary Internal Medicine (ACVIM) stage ($p < 0.001$), CKCS ($p = 0.005$), left atrium-to-aortic ratio (LA/Ao) ($p < 0.001$), E vel ($p < 0.001$) and $\log_{10}$(E/IVRT) ($p < 0.001$) were significant at the univariate level for PH development. At the multivariate level, only ACVIM stage remained significant ($p = 0.044$), suggesting that worsening MMVD was the predominant determinant of PH development in this study. Pulmonary hypertension was associated with greater likelihood of CHF ($p < 0.001$) and death (both cardiac [$p < 0.001$] and all-cause mortality [$p = 0.011$]). Cavalier King Charles spaniels were more likely to experience cardiac death than non-CKCS ($p = 0.004$).
Conclusions: In this study, development of PH was associated with worse MMVD, according to ACVIM stage.

Introduction

Myxomatous mitral valve disease (MMVD) is the most common acquired heart disease in dogs [1,2]. Cavalier King Charles spaniels (CKCS) are affected at an earlier age than other breeds [1,3—6]. Cavalier King Charles spaniels have been found to be more likely to die as a result of their heart disease [3,6—10], although one large study found that CKCS had a longer time to reach the endpoint (cardiac death) than non-CKCS [1,2,11].

Pulmonary hypertension (PH), defined in animals as pulmonary arterial systolic pressure greater than approximately 30 mmHg [1,3—6,12], is divided into five categories: 1) pulmonary arterial hypertension, 2) pulmonary hypertension due to increased left-sided filling pressures associated with left heart disease, e.g. MMVD, 3) pulmonary hypertension due to pulmonary disease and/or hypoxia, 4) pulmonary hypertension due to thromboembolic disease and 5) miscellaneous [3,6—10,12,13].

Right heart catheterization is the gold standard method of diagnosing PH but is rarely performed in clinical practice as it is invasive and requires sedation/anaesthesia [12]. Therefore, PH is most commonly diagnosed by measurement of the velocity of the tricuspid regurgitation (TR) jet, which can be used to determine the pressure gradient between the right ventricle and right atrium, via the modified Bernoulli equation (pressure gradient $= 4v^2$, where v is the velocity of the regurgitant jet). This provides an estimate of systolic pulmonary artery pressure [12], after exclusion of pulmonic stenosis. Diastolic pulmonary artery pressure is estimated in the same fashion, using the velocity of pulmonic regurgitation (PR) [12].

Left heart disease is one of the most common causes of PH in dogs [14]; the reported prevalence of PH in dogs with MMVD varies between 13 and 71% [15—19]. Increased left atrial pressure results in increased pulmonary venous and capillary pressures that result in elevated pulmonary arterial pressure [14]. This may be exacerbated by reactive, hypoxia-induced, pulmonary arterial vasoconstriction [14]. Dogs with asymptomatic MMVD are unlikely to have severe PH [1,2], and the severity and prevalence of PH increases with severity of MMVD [18,20] and is associated with a poorer prognosis [15,20]. It is not known why some dogs with MMVD develop PH and others do not.

To the authors' knowledge, there have been no studies showing an association between dog breed and development of PH secondary to MMVD. We hypothesized that CKCS with MMVD seen at our hospital had a greater prevalence of PH than dogs of other breeds with MMVD.

The primary aim of this study was to determine whether CKCS were more likely to develop PH than non-CKCS with MMVD and to explore which variables may be associated with development of PH.

As a secondary aim, we explored whether breed (CKCS yes/no) or the presence of PH impacted survival, for both all-cause and cardiac mortalities.

Materials and methods

The study was approved by the Veterinary Research Ethics Committee, University of Liverpool (reference: VREC503).

This was a retrospective data analysis project. The echocardiography analysis and archiving software[d] of the cardiology service of the Small Animal Teaching Hospital, University of Liverpool, was searched for dogs coded 'MMVD' (myxomatous mitral valve disease) over the period of 2008–2016. Echocardiography was performed by cardiology diplomates of the European College of Veterinary Internal Medicine (ECVIM) or residents in training working under their supervision. Measurements were taken offline by the diplomate/resident in question. An average of three consecutive R–R intervals was used for measurements (five if the patient was in atrial fibrillation [AF]). Patients were gently manually restrained in right and left lateral recumbency. All echocardiographies were performed without sedation. Dogs eligible for inclusion satisfied the following criteria: 1) aged five years or older, 2) body weight $<$20 kg, 3) echocardiographic diagnosis of MMVD, defined as nodular lesions on the mitral valve, with or without prolapse and mitral regurgitation on colour Doppler, 4) evidence of cardiac remodelling changes (ACVIM stage B2, C or D [21]). Left-sided CHF (L-CHF) was diagnosed based on clinical signs, the presence of pulmonary venous congestion and cardiogenic pulmonary oedema seen on thoracic radiographs and/or an improvement in clinical signs after furosemide administration in a patient with advanced MMVD seen echocardiographically. Right-sided CHF (R-CHF) was defined by the presence of ascites, pleural effusion or small-volume pericardial effusion in the absence of other conditions that may have been responsible. Patients were considered to be in stage D of their disease if they were refractory to high doses of furosemide (8–12 mg/kg/day) and had therefore been prescribed torasemide and/or hydrochlorothiazide. As all dogs had been referred by a primary veterinarian, some dogs had been treated with furosemide, benazepril and pimobendan to stabilize them before the initial echocardiography. Dogs without structural heart changes due to MMVD or without remodelling (i.e. ACVIM stages A and B1) and those with PH due to other causes were excluded. Dogs with other structural heart disease (e.g. congenital) were also excluded. Dogs with concurrent cardiac disease, either congenital or acquired (e.g. dilated cardiomyopathy and endocarditis), were excluded. Dogs with known concurrent respiratory disease seen on thoracic imaging (computed tomography, radiography or tracheobronchoscopy) (e.g. neoplasia, idiopathic pulmonary fibrosis and pneumonia) or significant systemic disease that may have affected pulmonary pressures (e.g. angiostrongylosis) were also excluded. Dogs were not excluded if they had AF as it was most likely to be a consequence of their MMVD. From the computerized hospital records[e], the following information was retrieved: date of birth, breed, gender, body weight and medications received. From the echocardiography records, the following data were obtained: left atrium-to-aortic ratio (LA/Ao) measured from the right parasternal short axis two-dimensional views at end diastole, optimizing LA size [22]; M-mode left ventricular internal diameter in diastole and systole, indexed for body weight by allometric scaling (LVIDDn and LVIDSn) [23]; TR velocity (from left apical view optimizing the right heart) and peak PR velocity (from either right or left cranial short axis views, whichever optimized the PR) [12,14]. Pulmonary hypertension was defined as TR $\geq$ 2.8 m/s with or without PR $\geq$ 2.2 m/s [12,14,15]. As an estimate of left-sided filling pressures, transmitral E wave velocity (E vel), isovolumic relaxation time and ratio of E wave to isovolumic relaxation time (E/IVRT) data were retrieved [12,19], having been acquired from left apical four and five chamber views. From images obtained from the right parasternal long axis, four-chamber view, optimizing left ventricular length and area, the end-systolic left ventricular volume, derived by Simpson's method of discs [24] indexed to body surface area, was retrieved to estimate left ventricular systolic function, with values $>$ 30 mL/m^2 considered to be consistent with systolic dysfunction [25,26]. For dogs with multiple echocardiographic examinations, the visit associated with the highest TR velocity was used.

Survival analysis

Where possible, outcome data were obtained from clinical records. Otherwise, primary veterinary practices were contacted. Death was defined as cardiac (euthanasia due to progressive cardiac disease or sudden cardiac death) or all-cause

[d] GE EchoPAC, version 113, GE Medical Systems, Buckinghamshire, UK.

[e] Tristan Veterinary Practice Management Solution, version 1.8.3.1110.

mortality (cardiac death plus death due to all other causes).

Statistical analysis

Statistical analysis was performed using commercially available software[f,g]. Normality of data distribution was determined using Shapiro–Wilk tests. Age in years and E vel within ACVIM subgroups B2 and C were normally distributed, were represented as mean (standard deviation) and were compared between CKCS/non-CKCS and PH/non-PH dogs using an independent *t*-test. There were only three non-CKCS stage D dogs, and therefore, statistical analysis was not performed on this group. Tricuspid regurgitation velocity was normally distributed and is represented as mean ± standard deviation and was compared across ACVIM classes of heart disease using a one-way analysis of variance with Bonferroni correction applied to assess statistical significance for post hoc analysis. No other data were normally distributed; therefore, continuous data (body weight, LA/Ao TR velocity for patients with PH, PR, E vel, E/IVRT, end-systolic volume index [ESVI], LVIDDn and LVIDSn) are represented as median [range] and were compared using a Mann–Whitney U test. Categorical data (gender, presence of PH [y/n], mean peak PR $>$ 2.2 m/s [y/n], breed = CKCS [y/n], presence of CHF [y/n], PH prevalence within ACVIM class and ESVI $>$ 30 mL/m^2) were compared using Chi-squared tests. The prevalence of AF between CKCS/non-CKCS and PH/non-PH dogs was determined using a Fishers exact test, due to low numbers. Univariate and multivariate analyses were performed using backwards logistic regression to assess for the effects of individual factors on the development of PH (age, breed [CKCS/non-CKCS], ACVIM stage, LA/Ao, E vel and $\log_{10}$ [E/IVRT]). Values that were significant at the univariate level ($p < 0.2$) were used in the multivariate model. Survival time was assessed on all-cause mortality and cardiac mortality using Kaplan Meier curves and log-rank analysis. A value of $p < 0.05$ was considered statistically significant.

Results

A total of 187 dogs met the inclusion criteria for the study. There were 94 CKCS and 93 non-CKCS (Table 1). The breeds represented in the non-CKCS group were 19 crossbreeds, 10 Chihuahuas, eight Cocker spaniels, six Yorkshire terriers, five Bichon frisés and four or fewer of the following: Miniature schnauzer, Japanese spitz, Border collie, whippet, Maltese, Jack Russell terrier, lurcher, Griffon Bruxellois, Norfolk terrier, Shetland sheepdog, dachshund, Airedale, Tibetan terrier, Lancashire heeler, Scottish terrier, Shih tzu, toy poodle, Staffordshire bull terrier, Lhasa apso, Irish terrier, King Charles spaniel, Hungarian hound, Pekingese and beagle. One hundred nine dogs were male, and 78 were female.

The rest of the signalment, key echocardiographic variables and presence of L-CHF data are shown in Table 1.

There was no difference in age, weight or gender between CKCS and non-CKCS. Eight dogs had AF at the time of inclusion in the study, three CKCS and five non-CKCS ($p = 1.00$).

The dogs were in different stages of their disease and as such were being treated with a variety of cardiac medications: furosemide (n = 66), pimobendan (77), spironolactone (51), benazepril (68), torasemide (2), hydrochlorothiazide-amiloride (5), digoxin (3), diltiazem (3), amlodipine (3), aspirin (2), clopidogrel (3), sildenafil (4), ramipril (1), codeine (1) and sotalol (4). Combinations of medications are provided in Supplemental Table A (available online). One hundred one dogs were not receiving any medications. Fourteen dogs were in R-CHF, 11 CKCS and three non-CKCS, with two crossbreed dogs and one Staffordshire bull terrier.

Left atrium-to-aortic ratio ($p = 0.036$), E vel ($p = 0.011$) and E/IVRT ($p = 0.01$) were significantly higher in CKCS (Table 1). E wave velocity and E/IVRT were not significantly different within ACVIM groups when comparing CKCS and non-CKCS.

Pulmonary hypertension was more prevalent in CKCS than non-CKCS ($p = 0.005$; Table 1). The dogs with PH had higher LA/Ao ($p < 0.001$), E wave velocity ($p < 0.001$) and E/IVRT ($p < 0.001$) than the dogs without PH (Table 2).

The prevalence of PH did not differ within ACVIM stage for CKCS/non-CKCS (Supplemental Table B, available online). All dogs in stage D (12 CKCS and three non-CKCS) had PH. Six of the eight dogs with AF had PH ($p = 1.00$). No dog had isolated diastolic PH (i.e. PR $>$ 2.2 m/s in the absence of TR $>$ 2.8 m/s).

The mean TR velocity across all dogs was 3.4 m/s and was significantly higher for CKCS (3.52 m/s [0.77–5.66 m/s]) than non-CKCS (3.25 m/s [1.54–4.68 m/s]; $p = 0.031$; Fig. 1). There was no difference in TR velocity within ACVIM stage between CKCS and non-CKCS (data not shown). Of the 102 dogs with L-CHF, 82 had PH

[f] SPSS Statistics, version 24, IBM.
[g] GraphPad Prism, version 7; GraphPad Software Inc.

Table 1 Population characteristics and echocardiographic variables of the 187 dogs in the study, comparing Cavalier King Charles spaniels (CKCS) and non-CKCS.

Parameter	All dogs	CKCS	Non-CKCS	p value (CKCS vs non-CKCS)
Number	187	94	93	–
Age in years (mean ± SD)	9.72 ± 2.32	9.72 ± 2.26	9.72 ± 2.39	0.992
Weight in kg (median [range])	10.2 [1.9–19.8]	10.2 [5.5–17.1]	10.2 [1.9–19.8]	0.397
Male (number [%])	109 [58.3%]	59 [62.8%]	50 [53.8%]	0.212
PH (number [%])	123 [65.8%]	71 [75.5%]	52 [55.9%]	**0.005**
LA/Ao (median [range])	1.94 [1.19–3.53]	2.03 [1.21–3.44]	1.91 [1.19–3.53]	**0.036**
TR velocity in m/s (median [range])	3.4 [0.77–5.66]	3.52 [0.77–5.66]	3.25 [1.54–4.68]	**0.031**
PR velocity in m/s (median [range]); data available for only 96	1.81 [0.31–3.8]	1.95 [0.31–3.8]	1.49 [0.69–3.25]	**0.01**
E vel in m/s (median [range])	1.17 [0.49–2.41]	1.24 [0.51–2.41]	1.06 [0.49–1.99]	**0.011**
E/IVRT (median [range])	1.89 [0.51–21.50]	2.13 [0.63–21.50]	1.67 [0.51–6.60]	**0.01**
ESVI in mL/m^2 (median [range]; data available for only 185 dogs)	26.8 [7.3–94.5]	27.1 [7.3–94.5]	24.9 [7.5–83.9]	0.151
ESVI > 30 m mL/m^2 (number [%]; data available for only 185 dogs)	74 [40%]	36 [39%]	38 [41%]	0.81
LVIDDn (median [range])	1.97 [1.32–2.99]	2.08 [1.48–2.99]	1.88 [1.48–2.69]	**< 0.001**
LVIDSn (median [range])	1.07 [0.48–1.88]	1.11 [0.48–1.88]	1.03 [0.58–1.64]	0.937
L-CHF (number [%])	103 [55.1%]	57 [60.6%]	46 [48.4%]	0.093

Figures in bold indicate statistical significance.
Abbreviations: E vel: transmitral E wave velocity, E/IVRT: ratio of E wave velocity to isovolumic relaxation time, ESVI: end-systolic volume index, LA/Ao: left atrium-to-aortic ratio, L-CHF: left-sided congestive heart failure, LVIDDn: left ventricular internal diameter in diastole, indexed for body weight, LVIDSn: left ventricular internal diameter in systole, indexed for body weight, PH: pulmonary hypertension, PR: pulmonic regurgitation, SD: standard deviation, TR: tricuspid regurgitation.

(80.1%), either at the time of the examination or previously diagnosed and controlled on medication. Twelve out of fourteen dogs with R-CHF had PH. The mean TR velocity was significantly lower in all dogs in stage B2 (mean: 2.84 ± 0.64 m/s) than in those in stage C (mean: 3.4 ± 0.72 m/s) or D (mean: 3.94 ± 0.75 m/s; $p < 0.001$ for both) and was lower in dogs in stage C than in those in stage D ($p = 0.023$ after correction) (Fig. 2).

Table 2 Population characteristics and echocardiographic variables of the 187 dogs in the study, comparing dogs with and without pulmonary hypertension (PH).

Parameter	All dogs	PH	Non-PH	p value (PH vs non-PH)
Number	187	123	64	–
Age in years (mean ± SD)	9.71 ± 2.32	9.61 ± 2.31	9.93 ± 2.34	0.365
Weight in kg (median [range])	10.2 [1.9–19.8]	10.1 [2.5–19.8]	10.65 [1.9–19.1]	0.342
LA/Ao (median [range])	1.94 [1.19–3.53]	2.09 [1.19–3.53]	1.74 [1.33–3.18	**< 0.001**
Male (number [%])	109 [58.3%]	71 [57.7%]	38 [59.4%]	0.828
E vel in m/s (median [range])	1.17 [0.49–2.41]	1.25 [0.49–2.41]	0.96 [0.51–1.64]	**< 0.001**
E/IVRT (median [range])	1.89 [0.51–21.50]	2.25 [0.51–21.50]	1.39 [0.54–4.46]	**< 0.001**
ESVI in mL/m^2 (median [range]; data available for only 185 dogs)	26.8 [7.3–94.5]	27.4 [7.3–94.5]	25.5 [7.5–56.1]	0.162
ESVI > 30 mL/m^2 (number [%]; data available for only 185 dogs)	74 [40%]	53 [44%]	21 [33%]	0.147
LVIDDn (median [range])	1.97 [1.32–2.99]	2.09 [1.32–2.99]	1.8 [1.48–2.43]	**< 0.001**
LVIDSn (median [range])	1.07 [0.48–1.88]	1.07 [0.48–1.88]	1.08 [0.64–1.41]	0.234
L-CHF (number [%])	103 [55.1%]	82 [66.7%]	20 [31.3%]	**< 0.001**

Figures in bold indicate statistical significance.
Abbreviations: E vel: transmitral E wave velocity, E/IVRT: ratio of E wave velocity to isovolumic relaxation time, ESVI: end-systolic volume index, LA/Ao: left atrium-to-aortic ratio, L-CHF: left-sided congestive heart failure, LVIDDn: left ventricular internal diameter in diastole, indexed for body weight, LVIDSn: left ventricular internal diameter in systole, indexed for body weight, PH: pulmonary hypertension, SD: standard deviation.

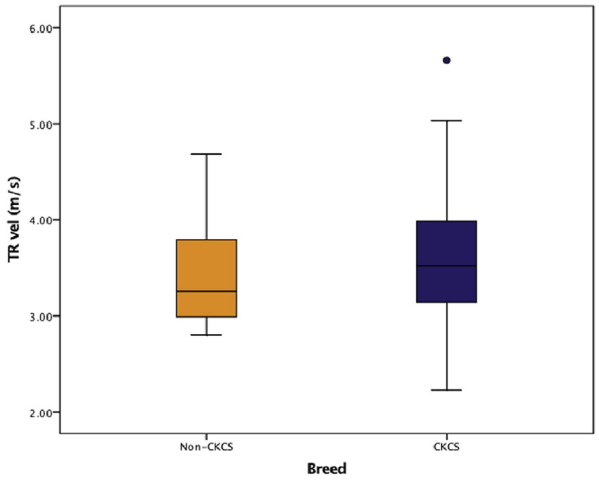

Fig. 1 Tricuspid regurgitation (TR) velocity compared between Cavalier King Charles spaniels (CKCS) and non-CKCS. The CKCS group had a significantly higher TR velocity (3.52 m/s [0.77—5.66 m/s]) than the non-CKCS group (3.25 m/s [1.54—4.68 m/s]).

Pulmonic regurgitation could be measured in 96 dogs; 55 CKCS and 41 non-CKCS. The median PR velocity was higher in CKCS ($p = 0.01$; Table 1). Twenty-four CKCS and six non-CKCS had peak PR > 2.2 m/s ($p = 0.02$); all these patients had systolic PH as well.

A total of 74 out of 185 (40%) dogs had reduced systolic function, as defined by an ESVI > 30 mL/m^2. There was no difference in the ESVI between CKCS/non-CKCS ($p = 0.151$; Table 1) or PH/non-PH dogs ($p = 0.162$; Table 2), and the number of dogs with systolic dysfunction did not differ across groups (Tables 1 and 2). Left ventricular internal diameter in diastole, indexed for body weight, was greater in both CKCS and dogs with PH ($p < 0.001$ for both), whereas LVIDSn did not differ between CKCS/non-CKCS ($p = 0.937$) or PH/non-PH dogs ($p = 0.234$; Tables 1 and 2).

Logistic regression found ACVIM stage ($p < 0.001$), CKCS ($p = 0.005$), LA/Ao ($p < 0.001$), E vel ($p < 0.001$) and $\log_{10}$(E/IVRT) ($p < 0.001$) to be significant at the univariate level for the development of PH. At the multivariate level, only ACVIM stage remained significant ($p = 0.044$).

Survival analysis

A total of 130 out of 187 dogs were dead at the time the study was conducted (all-cause mortality). There was no difference in all-cause mortality between CKCS and non-CKCS ($p = 0.086$; Fig. 3). Dogs with PH were more likely to die than those without PH ($p = 0.011$; Fig. 4).

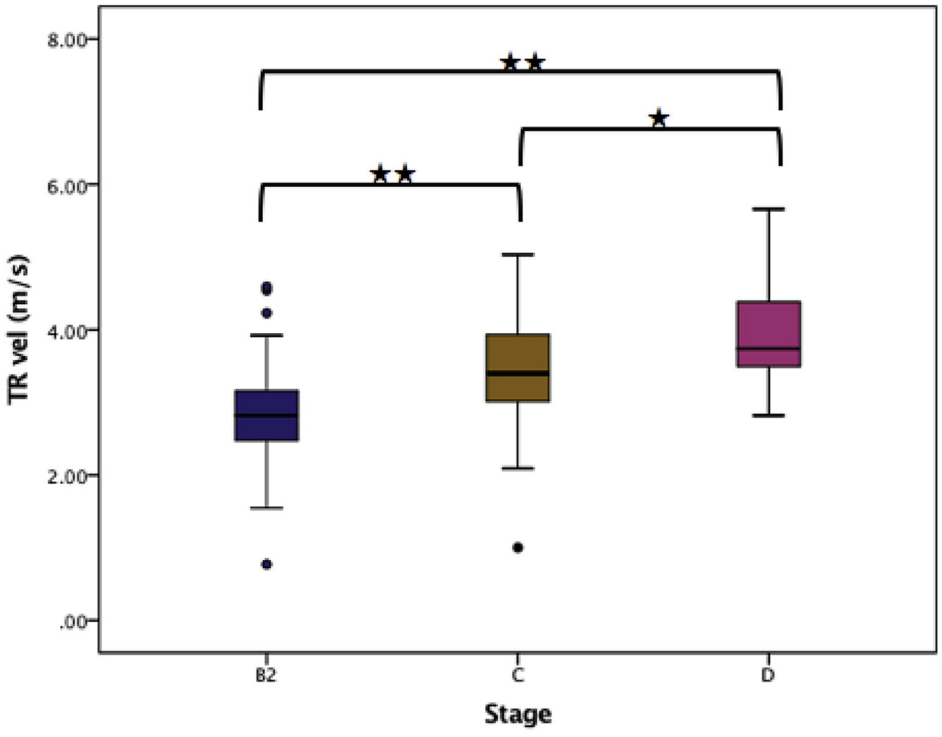

Fig. 2 Tricuspid regurgitation velocity across ACVIM stage. Statistical significance between groups is symbolized as: ★ $p < 0.05$; ★★ $p < 0.001$. ACVIM, American College of Veterinary Internal Medicine; TR, tricuspid regurgitation.

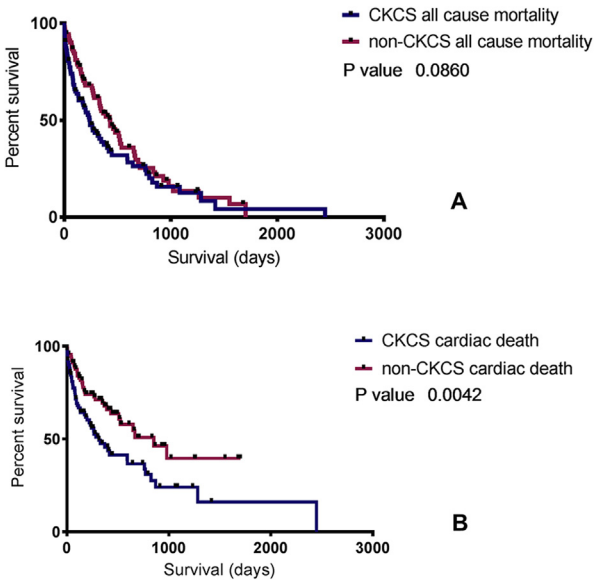

Fig. 3 Kaplan–Meier curves to compare survival between Cavalier King Charles spaniels (CKCS) and non-CKCS. A: All-cause mortality: CKCS median survival, 240 days; non-CKCS median survival, 430 days. B: Cardiac mortality: CKCS median survival, 313 days; non-CKCS median survival, 852 days.

Eighty-seven dogs died or were euthanized due to cardiac disease: CKCS were significantly more likely to die a cardiac death ($p = 0.004$; Fig. 3). Dogs with PH were significantly more likely to die a cardiac death ($p < 0.001$; Fig. 4); dogs without PH were unlikely to experience a cardiac death, with fewer than 50% reaching this endpoint.

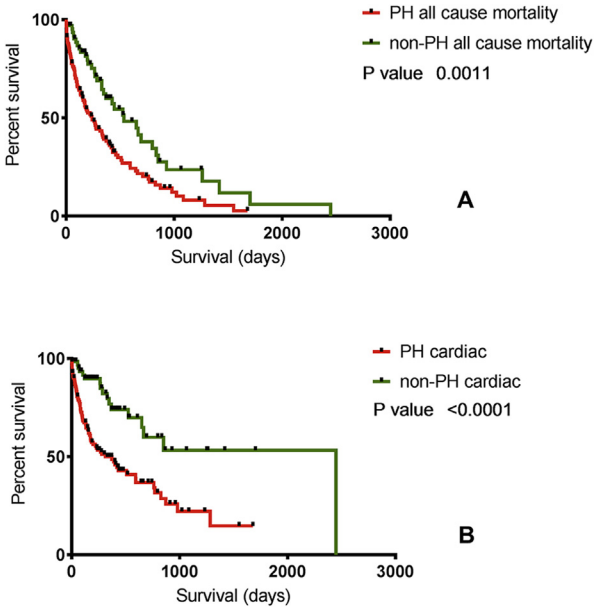

Fig. 4 Kaplan–Meier curves to compare survival between patients with pulmonary hypertension (PH) and non-PH patients. A: All-cause mortality: PH median survival, 240 days; non-PH median survival, 535 days. B: Cardiac mortality: PH median survival, 313 days; median survival not calculated for non-PH dogs as event rate <50%.

A total of 43 out of 187 dogs died or were euthanized due to non-cardiac disease. Causes of death included neoplasia (lymphoma, mast cell tumour, haemangiosarcoma and mass of unconfirmed diagnosis), chronic kidney disease, neurological disease (seizures and intervertebral disc disease), gastrointestinal disease (chronic diarrhoea and pancreatitis), intra-operative/postoperative complications, immune-mediated haemolytic anaemia or a combination of multiple comorbidities. For 12 patients, the cause of death was not clear from communications with the owners or referring veterinary surgeon.

Discussion

In this study, univariate analysis indicated a positive association between breed (CKCS) and development of PH. However, breed did not remain significant at the multivariate level. Multivariate logistic regression showed ACVIM stage to be the primary determinant in the development of PH in dogs with MMVD, suggesting that these patients had postcapillary PH, at least predominantly, due to chronically elevated left atrial pressures [12,14]. This is in accordance with previous findings [18,20]. It may be that LA/Ao and E/IVRT were 'intervening variables', i.e. related causally to the process by which disease severity causes PH. Progression from stage B2 to C to D by definition requires development of increased LA size and pressure [2,12,19], which is a key determinant in the development of postcapillary PH [12–14]. Patients that have progressed to stage C and D have been exposed to greater LA pressures for a prolonged period of time, which would allow more time for vascular remodelling and PH development [14].

There were more stage D CKCS than non-CKCS, which may in part explain the greater prevalence of PH among CKCS in this population. It is not clear from this study if the higher number of stage D CKCS represents a propensity for the breed to progress more rapidly to severe stages of the disease and die earlier, which would be in agreement with the findings by Serfass et al. [8]. Alternately, CKCS have a longer overall survival or are better able to tolerate severe CHF and are therefore more likely to gradually develop CHF that is refractory to high furosemide doses. The latter theory is potentially in agreement with the findings of the QUEST study [11], which found that CKCS

had a longer time to reach the endpoint of their study (cardiac death, euthanasia due to CHF or treatment failure). In our study population, all cases were not enrolled in this study at the time of MMVD diagnosis but the point at which their TR velocity was greatest, and as such, we cannot comment on the overall disease progression time.

The LA/Ao was significantly larger in CKCS than non-CKCS, which may in part explain why neither breed nor LA/Ao was significant at the multivariate level for PH development. It may be due to the fact that there were more stage D CKCS than non-CKCS. The LA/Ao was also significantly larger in dogs with PH than in those without PH.

The prevalence of PH in this study of dogs with MMVD was 65.8%, which is in keeping with previously published findings [15—17,19]. It should be noted that the present study excluded dogs with mild MMVD (stage B1), which was not the case in some other studies; therefore, these studies may have had a lower prevalence by virtue of having relatively fewer patients with more advanced disease. For example, Serres et al. [18] found PH in 13.9% of patients with MMVD across all International Small Animal Cardiac Health Council categories of heart disease severity.

Cavalier King Charles spaniels overall had a higher median E/IVRT; however, within a given ACVIM class, this was not the case, suggesting that dogs at the same disease stage had similar filling pressures. The high number of CKCS in stage D is likely responsible for the overall difference in E/IVRT between the groups. The differences in echocardiographic findings in CKCS compared to non-CKCS may reflect the unique natural history and disease progression seen in this breed [3,15—19,28], which has been hypothesized to be reflected in their different outcomes in numerous veterinary trials. For example, CKCS have previously been shown to develop clinical disease at an early age [3,14,28,29].

It is possible that undiagnosed concurrent disease (for example, respiratory conditions such as bronchomalacia, pulmonary vascular disease or pulmonary thromboembolism, which are common in small breed MMVD-prone dogs [12,15,20,30,31]) may have contributed to PH (combined postcapillary and precapillary PH) [13]. Precapillary and postcapillary PH are differentiated by measuring pulmonary capillary wedge pressure, an approximation of pulmonary venous and left atrial pressure, which is increased in postcapillary PH [12,13]. This requires right heart catheterization, an invasive procedure that is not routinely performed in small animals. In human medicine, echocardiographic variables and ratios, such as the recently coined 'echocardiographic pulmonary to left atrial ratio' [32], have been proposed as a non-invasive surrogate for differentiating between precapillary and postcapillary PH; however, this has not been evaluated in dogs.

Dogs with PH were more likely to be in L-CHF. This is to be expected, as increasing LA pressures are associated with both PH and L-CHF. It should, however, be noted that 40 (33%) dogs with PH did not have overt CHF, i.e. were in stage B2. Previous studies have found a PH prevalence of 19.9—47% in asymptomatic (either ISACHC class II or ACVIM stage B2) dogs with MMVD [18,20]. In this population, the B2 dogs with PH comprised of 22 CKCS (59.4%) and 18 non-CKCS (38.3%), with possible over-representation of B2 CKCS with PH, although this did not achieve statistical significance.

Tricuspid regurgitation velocity, and therefore, systolic PH severity, increased with MMVD severity, in terms of ACVIM class. Dogs in stage B2 had lower TR velocities than those in stage C, who in turn had lower TR velocities than stage D dogs. This finding is in agreement with other published literature showing a correlation between PH severity and heart disease severity [18,20].

End-systolic volume index and LVIDSn were not significantly different between CKCS and non-CKCS or between dogs with and without PH, suggesting that left ventricular systolic function did not differ significantly between those groups (although both the CKCS group and the PH group had larger LVIDDn). Systolic function is difficult to quantify in dogs with MMVD because the reduced afterload provided by the mitral regurgitation allows for normal or even hyperkinetic measurements of traditional systolic function variables, such as fractional shortening or ejection fraction. End-systolic volume index has been found to be increased in dogs with CHF due to MMVD [25,26]. Using ESVI >30 mL/m^2 as a cut-off, 74 dogs in this study had systolic dysfunction. There was no difference in the number of CKCS/non-CKCS or PH/non-PH dogs with systolic dysfunction, suggesting that LV systolic function is not associated with the development of PH in dogs with MMVD.

With regards to the survival analysis, it is important to remember that the starting point of the study was the time at which the highest TR value was documented. Therefore, the time to death was not from the first documented instance of PH but from its peak severity. This study was not designed to thoroughly evaluate the progression of PH and its effects on survival but more to observe the progression from the point at which PH was most severe echocardiographically. Furthermore, some patients were lost to follow-up for survival

analysis. Survival analysis was a secondary aim of the study; the authors primarily sought to establish whether prevalence of PH was greater for CKCS than dogs of other breeds.

Dogs with PH were more likely to experience cardiac or all-cause death. Pulmonary hypertension has been found to be a poor prognostic indicator in dogs with and without MMVD, particularly if the TR pressure gradient exceeds 55 mmHg [11,16,33—35].

In this study, CKCS were more likely to experience a cardiac death than non-CKCS; there was no difference in all-cause mortality between the two groups. This is in contrast to studies by Häggström et al. [11] and Pouchelon et al. [36], both of which reported a longer survival time in the CKCS group.

This was a retrospective study and is therefore subject to the limitations inherent to such studies. There was no standardization with regards to case assessment, diagnostic investigations or treatment. For example, there was no strict furosemide cut-off dose before switching to torasemide; in the hospital, torasemide is administered in patients already receiving 8—12 mg/kg of furosemide per day. Pre-existing medications may have affected echo measurements, in particular furosemide, pimobendan and sildenafil. Interobserver and intra-observer variations for echocardiographic measurements were not assessed. The study was conducted in a referral population and therefore may not represent the general population as a whole. Although efforts were made to exclude concurrent systemic or other respiratory disease, it is possible that some cases had underlying conditions that were undiagnosed, e.g. neoplasia, thromboembolic disease, angiostrongylosis, idiopathic fibrosis, etc. [12,14,15,35]. Having said that, the fact that PH prevalence and severity increased with ACVIM stage, along with the strong association with MMVD severity markers (LA/Ao, E velocity, E/IVRT), supports our presumption that MMVD contributed significantly to the development of PH. Pulmonary hypertension was not diagnosed by direct pulmonary arterial catheterization. This is considered the gold standard; however this is not routinely used in veterinary medicine because it is invasive, expensive and requires general anaesthesia. Tricuspid regurgitation velocity is used as standard by veterinarians to diagnose PH [12]. Velocities can be underestimated due to imperfect alignment of the Doppler cursor with the flow of the regurgitant jet.

Similarly, 12 of 14 dogs in R-CHF had PH; PH is known to predispose to R-CHF development [2,14], and these patients would have had increased right atrial pressure and possibly reduced right ventricular systolic function, which would have reduced the TR velocity, leading to underestimation of the PH [14,27]. However, one study has found that adding estimated right atrial pressures is of little additional value [37]. In addition, sometimes, TR/PR are not present and therefore cannot be measured [38]. Right ventricular systolic function was not routinely assessed (e.g. by tricuspid annular plane systolic excursion [39]) in these patients. Eight patients had AF, which may have affected the measurement of the E vel, as the values can be more variable. This should be mitigated as we performed more measurements on dogs with AF (five rather than three) to obtain mean values.

Conclusions

In this study population of dogs with MMVD, CKCS were more likely to have PH than non-CKCS, although multiple logistic regression indicated that the only significant determinant of PH was ACVIM stage of disease, i.e. severity of MMVD and chronicity of elevated LA pressures. Further studies are needed to determine the causative mechanism for PH development in CKCS with MMVD. In this study population, CKCS were found to be more likely to experience a cardiac death than non-CKCS. Dogs with PH were more likely to die, both in terms of all-cause mortality and cardiac death.

Conflicts of Interest Statement

The authors do not have any conflicts of interest to disclose.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jvc.2019.01.002.

References

[1] Buchanan JW. Chronic valvular disease (endocardiosis) in dogs. Adv Vet Sci Comp Med 1977;21:75—106.

[2] Gordon SG, Saunders AB, Wesselowski SR. Asymptomatic canine degenerative valve disease. Vet Clin Nutr: Syst Pract 2017;29:1—21.
[3] Beardow AW, Buchanan JW. Chronic mitral valve disease in Cavalier King Charles spaniels: 95 cases (1987-1991). J Am Vet Med Assoc 1993;203:1023—9.
[4] Lewis T, Swift S, Woolliams JA, Blott S. Heritability of premature mitral valve disease in Cavalier King Charles spaniels. Vet J 2011;188:73—6.
[5] Swift S. The problem of inherited diseases. 5: valvular disease in Cavalier King Charles spaniels. J Small Animal Pract 1996;37:505—6.
[6] Pedersen D, Lorentzen KA, Kristensen BO. Echocardiographic mitral valve prolapse in Cavalier King Charles spaniels: epidemiology and prognostic significance for regurgitation. Vet Rec 1999;144:315—20.
[7] López-Alvarez J, Elliott J, Pfeiffer D, Chang Y-M, Mattin M, Moonarmart W, Hezzell MJ, Boswood A. Clinical severity score system in dogs with degenerative mitral valve disease. J Vet Intern Med 2015;29:575—81.
[8] Serfass P, Chetboul V, Sampedrano CC. Retrospective study of 942 small-sized dogs: prevalence of left apical systolic heart murmur and left-sided heart failure, critical effects of breed and sex. J Vet Cardiol 2006;8:11—8.
[9] Mattin MJ, Boswood A, Church DB, López-Alvarez J, McGreevy PD, O'Neill DG, Thomson PC, Brodblelt DC. Prevalence of and risk factors for degenerative mitral valve disease in dogs attending primary-care veterinary practices in England. J Vet Intern Med 2015;29:847—54.
[10] Swift S, Baldin A, Cripps P. Degenerative valvular disease in the Cavalier king Charles spaniel: results of the UK breed scheme 1991-2010. J Vet Intern Med 2017;31:9—14.
[11] Haggstrom J, Boswood A, O'Grady M, Jons O, Smith S, Swift S, Borgarelli M, Gavaghan B, Kresken J-G, Patteson M, Ablad B, Bussadori CM, Glaus T, Kovacevic A, Rapp M, Santilli RA, Tidholm A, Eriksson A, Belanger MC, Deinert M, Little CJL, Kvart C, French A, Rønn-Landbo M, Wess G, Eggertsdottir AV, O'Sullivan ML, Schneider M, Lombard CW, Dukes-McEwan J, Willis R, Louvet A, Difruscia R. Effect of pimobendan or benazepril hydrochloride on survival times in dogs with congestive heart failure caused by naturally occurring myxomatous mitral valve disease: the QUEST Study. J Vet Intern Med 2008;22:1124—35.
[12] Kellihan HB, Stepien RL. Pulmonary hypertension in dogs: diagnosis and therapy. Vet Clin North Am Small Anim Pract 2010;40:623—41.
[13] Galiè N, Humbert M, Vachiery J-L, Gibbs S, Lang I, Torbicki A, Simmoneau G, Peacock A, Vonk Noordefraaf A, Beghetti M, Ghofrani A, Gomez Sanchez MA, Hansmann G, Klepetko W, Lancellotti, Matucci M, McDonagh T, Pierard LA, Trindade PT, Zompatori M, Hoeper M. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension. Eur Heart J 2015 Dec 28;37:67—119.
[14] Kellihan HB, Stepien RL. Pulmonary hypertension in canine degenerative mitral valve disease. J Vet Cardiol 2012;14:149—64.
[15] Johnson L, Boon J, Orton EC. Clinical characteristics of 53 dogs with Doppler-derived evidence of pulmonary hypertension: 1992-1996. J Vet Intern Med 1999;13:440—7.
[16] Kellum HB, Stepien RL. Sildenafil citrate therapy in 22 dogs with pulmonary hypertension. J Vet Intern Med 2007;21:1258—64.
[17] Serres F, Chetboul V, Gouni V, Tissier R, Sampedrano CC, Pouchelon JL. Diagnostic value of echo-Doppler and tissue Doppler imaging in dogs with pulmonary arterial hypertension. J Vet Intern Med 2007;21:1280—9.
[18] Serres FJ, Chetboul V, Tissier R, Carlos Sampedrano C, Gouni V, Nicolle AP, Pouchelon J-L. Doppler echocardiography—derived evidence of pulmonary arterial hypertension in dogs with degenerative mitral valve disease: 86 cases (2001—2005). J Am Vet Med Assoc 2006;229:1772—8.
[19] Schober KE, Hart TM, Stern JA, Li X, Samii VF, Zekas LJ, Scansen BA, Bonagura J. Detection of congestive heart failure in dogs by Doppler echocardiography. J Vet Intern Med 2010;24:1358—68.
[20] Borgarelli M, Abbott J, Braz-Ruivo L, Chiavegato D, Crosara S, Lamb K, Ljungvall I, Poggi M, Santilli RA. Haggstrom. Prevalence and prognostic importance of pulmonary hypertension in dogs with myxomatous mitral valve disease. J Vet Intern Med 2015;29:569—74.
[21] Atkins C, Bonagura J, Ettinger S, Fox P, Gordon S, Haggström J, Hamlin R, Keene B, Luis Fuentes V, Stepien R. Guidelines for the diagnosis and treatment of canine chronic valvular heart disease. J Vet Intern Med 2009;23:142—50.
[22] Haggström J, Hansson K, Karlberg BE, Kvart C, Olsson K. Plasma concentration of atrial natriuretic peptide in relation to severity of mitral regurgitation in Cavalier King Charles Spaniels. Am J Vet Res 1994;55:698—703.
[23] Cornell CC, Kittleson MD, Torre Della P, Häggström J, Lombard CW, Pedersen HD, Vollmar A, Wey A. Allometric scaling of M-mode cardiac measurements in normal adult dogs. J Vet Intern Med 2004;18:311—21.
[24] Chetboul V, Tissier R. Echocardiographic assessment of canine degenerative mitral valve disease. J Vet Cardiol 2012;14:127—48.
[25] Borgarelli M, Tarducci A, Zanatta R, Häggström J. Decreased systolic function and inadequate hypertrophy in large and small breed dogs with chronic mitral valve insufficiency. J Vet Intern Med 2007;21:61—7.
[26] Bonagura JD, Schober KE. Can ventricular function be assessed by echocardiography in chronic canine mitral valve disease? J Small Anim Pract 2009;50:12—24.
[27] Baron Toaldo M, Poser H, Menciotti G, Battaia S, Contiero B, Cipone M, Diana A, Mazzotta E, Guglielmini. Utility of tissue Doppler imaging in the echocardiographic evaluation of left and right ventricular function in dogs with myxomatous mitral valve disease with or without pulmonary hypertension. J Vet Intern Med 2016;30:697—705.
[28] Atkins CE, Keene BW, Brown WA, Coats JR, Crawford MA, DeFrancesco TC, Edwards NJ, Fox PR, Lehmkuhl LB, Luethy MW, Meurs KM, Petrie J-P, Pipers FS, Rosenthal SL, Sidley JA, Straus JH. Results of the veterinary enalapril trial to prove reduction in onset of heart failure in dogs chronically treated with enalapril alone for compensated, naturally occurring mitral valve insufficiency. J Am Vet Med Assoc 2007;231:1061—9.
[29] Kvart C, Häggström J, Pedersen HD, Hansson K, Eriksson A, Järvinen A-K, Tidholm A, Bsenko K, Ahlgren E, Ilves M, Ablad B, Falk T, Bjerkfas E, Gundler S, Lord P, Wegeland G, Adolfsson E, Corfitzen. Efficacy of enalapril for prevention of congestive heart failure in dogs with myxomatous valve disease and asymptomatic mitral regurgitation. J Vet Intern Med 2002;16:80—8.
[30] Singh MK, Johnson LR, Kittleson MD, Pollard RE. Bronchomalacia in dogs with myxomatous mitral valve degeneration. J Vet Intern Med 2012;26:312—9.
[31] Bach JF, Rozanski EA, MacGregor J, Betkowski JM, Rush JE. Retrospective evaluation of sildenafil citrate as a therapy

for pulmonary hypertension in dogs. J Vet Intern Med 2006; 20:1132—5.
[32] Scalia GM, Scalia IG, Kierle R, Beaumont R, Cross DB, Feenstra J, Burstow DJ, Fitzgerald BT, Platts DG. ePLAR - the echocardiographic pulmonary to left atrial ratio - a novel non-invasive parameter to differentiate pre-capillary and post-capillary pulmonary hypertension. Int J Cardiol 2016;212:379—86.
[33] Luis Fuentes V. Use of pimobendan in the management of heart failure. Vet Clin Nutr: SA Pract 2004;34:1145—55.
[34] Haggström J, Lord PF, Hoglund K, Ljungvall I, Jöns O, Kvart C, Hansson K. Short-term hemodynamic and neuro-endocrine effects of pimobendan and benazapril in dogs with myxomatous mitral valve disease and congestive heart failure. J Vet Intern Med 2013;27:1452—62.
[35] Borgeat K, Sudunagunta S, Kaye B, Stern J, Luis Fuentes V, Connolly DJ. Retrospective evaluation of moderate-to-severe pulmonary hypertension in dogs naturally infected with Angiostrongylus vasorum. J Small Animal Pract 2015; 56:196—202.
[36] Pouchelon JL, Jamet N, Gouni V, Tissier R, Serres F, Carlos Sampedrano C, Castaignet M, Lefebvre HP, Chetboul V. Effect of benazepril on survival and cardiac events in dogs with asymptomatic mitral valve disease: a retrospective study of 141 cases. J Vet Intern Med 2008;22:905—14.
[37] Soydan LC, Kellihan HB, Bates ML, Stepien RL, Consigny DW, Bellofiore A, Francois CJ, Chesler NC. Accuracy of Doppler echocardiographic estimates of pulmonary artery pressures in a canine model of pulmonary hypertension. J Vet Cardiol 2015;17:13—24.
[38] Tidholm A, Hoglund K, Haggström J, Ljungvall I. Diagnostic value of selected echocardiographic variables to identify pulmonary hypertension in dogs with myxomatous mitral valve disease. J Vet Intern Med 2015;29:1510—7.
[39] Pariaut R, Saelinger C, Strickland KN, Beaufrère H, Reynolds CA, Vila J. Tricuspid annular plane systolic excursion (TAPSE) in dogs: reference values and impact of pulmonary hypertension. J Vet Intern Med 2012;26: 1148—54.