Original Article

Intermittent mitral regurgitation in Cavalier King Charles spaniels: Short-term progression and influence of stress tests

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Highlights

- Stress tests reduced the presence of intermittent mitral regurgitation (iMR)
- iMR was not present at mean heart rates above 150 beats/min
- Heart rate response was similar for dogs with and without iMR
- iMR did not predict short-term progression of mitral regurgitation

Abstract

In young Cavalier King Charles spaniels (CKCS), intermittent mitral regurgitation (iMR; defined as moderate to severe mitral regurgitation [MR] in a small proportion of heartbeats), has been associated with an increased risk of cardiac death due to myxomatous mitral valve disease (MMVD). It is associated with increased R-R interval variability. Little is known about response to physiological factors and whether iMR is a precursor for developing significant MR. The aim of this study was to determine the effect of stress testing on the presence of iMR and heart rate, and short-term (1-2 year) progression of MR in CKCS with and without iMR. In total, 52 CKCS
were included. Substudy 1 enrolled six dogs with iMR and 11 dogs without iMR. Substudy 2 enrolled 14 dogs with iMR and 28 dogs without iMR. Substudy 1 prospectively assessed the influence of stress testing on the presence of iMR and heart rate. Substudy 2 retrospectively evaluated short-term progression of iMR.

During stress testing, iMR disappeared in 50% of CKCS and no iMR was recorded at mean heart rates >150 beats/min. Heart rate response did not differ between CKCS with or without iMR. CKCS with iMR did not have a higher odds (odds ratio=5.2; 95% confidence interval, 0.7-38.2) of MR progression compared to controls (P = 0.1). In conclusion, physical stress influenced the occurrence of iMR in CKCS, but heart rate response was not different from CKCS without iMR. Intermittent mitral regurgitation did not significantly predict short-term MR progression. In stressed CKCS with early disease, iMR may be overlooked.

Keywords: Dog; Myxomatous mitral valve disease; R-R interval variability; Sinus arrhythmia; Stress test

Introduction

Pronounced sinus arrhythmia (SA) has been associated with mitral valve prolapse (MVP) in dogs with early stages of myxomatous mitral valve disease (MMVD; Pedersen et al., 1995, 1996; Olsen et al., 1999;). It can be difficult to differentiate SA from atrial premature complexes (APCs) in dogs, because APCs can have normal P waves (Kittleson and Kienle, 1998). An association between APCs and MMVD severity in dogs is undetermined (Crosara et al., 2010; Rasmussen et al., 2012). In the late MMVD disease stages with congestive heart
failure (CHF), SA usually ceases as tachycardia develops, possibly as a result of increased sympathetic tone and neurohormonal activation (Häggeström et al., 1996; Lopez-Alvarez et al., 2014; Rasmussen et al., 2012 and 2014). The same is observed in humans with CHF where reduced heart rate variability is associated with adverse outcome (Jiang et al., 1997) and recent data suggest that reduced SA even may aid early recognition of CHF (Patel et al., 2017).

In some Cavalier King Charles spaniels (CKCS) with MMVD, pronounced SA and/or APCs have been associated with the presence of intermittent mitral regurgitation (iMR), and iMR appears in the systole of long R-R intervals when they follow a short R-R interval in these dogs (Reimann et al., 2014b). Interestingly, iMR in young CKCS has been shown to predict cardiac death later in life (Reimann et al., 2017b). Short-term progression of iMR has not previously been determined.

It could be hypothesized that dogs with iMR have a different autonomic regulation of the heart compared to dogs without iMR due to the association between iMR and R-R interval variability. An autonomic dysfunction is described in some human patients with mitral valve prolapse (Boudoulas et al., 1983). Some humans with MVP have a different heart rate response to adrenergic stimuli (Orhan et al., 2009). Furthermore, stimulation of valvular adrenergic and cholinergic nerves can influence valvular smooth muscle activity in humans (Curtis and Priola, 1992). Evidence of autonomic nerve activity has also been demonstrated in canine papillary muscle (Cronin et al., 1969; Endoh and Hashimoto, 1970). In theory, modified autonomic tonus might lead to a valvular and/or papillary muscle dysfunction and thereby iMR. However, the etiology and impact of physiologic factors on iMR are unknown in CKCS.
An important part of an echocardiographic examination is an assessment for the presence of MR (Keene et al., 2019). Echocardiographic examinations normally are performed on unsedated, restrained dogs, which causes increased stress levels and high heart rates in some dogs. The effects of stress and increased heart rate on iMR have not been studied previously. Furthermore, it has not been investigated if dogs with iMR respond differently to stressful stimuli compared to dogs without iMR, which may indicate an autonomic dysfunction in dogs with iMR. Because an increased heart rate will decrease R-R interval variability (Häggström et al., 1996), it could be hypothesized that an iMR would disappear during stress tests (Reimann et al., 2014b). Hence, it is possible that an increased heart rate may influence the echocardiographic evaluation of MR severity and could result in undiagnosed MR.

The aims of this study were to investigate effect of a stress tests on: (1) presence of iMR; (2) heart rate in dogs; and (3) the short-term (1-2 year) progression of iMR in CKCS. We hypothesized that: (1) stress tests would reduce the presence of iMRs; (2) stress tests would result in a smaller increase in heart rate in CKCS with iMR compared to CKSC without iMR; and (3) a higher proportion of CKCS with iMR would develop more severe disease (constant MR≥20%) than CKCS without iMR within 1-2 years.

Materials and methods

This study was performed at the Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark and approved by the Danish Animal Experiments Inspectorate (License number: 2006/561-1145, Approval date: 10th May 2006; License number: 2012-15-2934-00700, Approval date: 29th September 2011; and License number: 2016-15-0201-
01074, Approval date: 3rd November 2016). All dog owners agreed to participate by written informed consent. CKCS were examined using a standardized protocol including: interview with the owner, physical examination, and echocardiography. Left apical systolic heart murmur intensity was graded 1-6/6 (Gompf, 1988). Study groups were defined as follows: control, MR <20%; iMR, MR <20% in majority of cardiac systoles, but some MR jets ≥20%; constant MR, MR ≥20%.

Substudy 1

This prospective study included client-owned CKCS that were invited to participate if the CKCS had been examined within the last 1.5 years or during the recruitment period and with MR that fitted into the control or iMR study groups. The examinations were performed between September 2014 to December 2014. Because of the descriptive, exploratory nature of this substudy and because no research data describing iMR during stress testing were available, no sample size calculation was performed before the start of the study. Exclusion criteria included pregnancy or lactation, significant systemic or organ related disease and cardiac disorders other than MMVD. Previous studies regarding different research questions included selected data from 10 of the CKCS in this substudy (Meurs et al., 2018; Christiansen et al., 2019).

Echocardiographic assessment

All echocardiographic examinations were performed by one of two operators (substudy 1, LHO; substudy 2, LHO or MJR) and offline evaluation was performed by one operator (MJR). During the echocardiographic offline evaluation, the operator was blinded to the identity and clinical data of the dog. The transthoracic echocardiographic examination (Vivid...
echocardiograph with 3S and 5S transducers [2011–2014] and VividE9 echocardiograph with a 5Sc transducer [2014-2017]; GE Healthcare Danmark A/S from parasternal and apical windows; Thomas et al., 1993) followed a standardized protocol. Continuous electrocardiographic (ECG) monitoring was used to assess heart rate during the echocardiographic examination.

Severity of MR assessed from the left apical 4-chamber view by 2D color Doppler flow mapping was used to allocate dogs into groups based on regurgitant jet area relative to left atrial area (Pedersen et al., 1999). Left atrial-to-aortic root ratio (LA/Ao) was determined from the 2D right parasternal short axis view at the level of the aortic root (Häggström et al., 1994). M-mode short axis images were used to determine left ventricular (LV) end-systolic and diastolic diameters and fractional shortening (FS; Lombard, 1984). The LV diameters were normalized to BW (Cornell et al., 2004). Right sided long axis LV end-systolic and end-diastolic volumes were obtained by tracing of the endocardium from right parasternal and left apical 4-chamber views and ejection fraction (EF) was calculated (Wess et al., 2010). End-systole was defined as the frame before mitral valve opening and end-diastole as the frame before mitral valve closure.

Severity of MMVD was staged according to American College of Veterinary Internal Medicine (ACVIM) consensus statement guidelines as follows: group A, CKCS with no auscultatory heart murmur and normal echocardiogram (MR < 20%); group B1, CKCS with auscultatory heart murmur or MR ≥ 20% and LA/Ao < 1.6 or left ventricular end diastolic diameter normalized for bodyweight (LVIDDN) < 1.7; group B2, CKCS with ≥ grade 3 heart murmur and LA/Ao ≥ 1.6 and LVIDDN ≥ 1.7 (Boswood et al., 2016; Keene et al., 2019).

The last part of the echocardiographic examination included stress tests to increase heart rate with assessment of MR from the left apical 4-chamber view by 2D color Doppler flow.
mapping at baseline and during each stressful stimulus (stress). The CKCS were exposed to three different stressful stimuli (stress tests) in the following order: (1) the sound of a squeaky toy squeezed four times; (2) the sound of a recorded meowing cat; and (3) a short run of approximately 40m. Before each stressful stimulus, a baseline was recorded. Baselines 2 and 3 were recorded when an iMR was seen (iMR group) or when the heart rate was lower than the maximal heart rate at baseline 1 (control group). On all baseline and stress recordings, the presence of iMR, mean and maximal heart rates were noted. Mean heart rates were calculated as an average of the values measured by the ultrasound system for each RR interval. Baseline recordings included 10 R-R intervals and stress recordings included 20 R-R intervals (the first 10 R-R intervals after the sound was initiated and the last 10 R-R intervals after the sound was terminated, or 20 cycles after the run).

Substudy 2

This study included client-owned CKCS that had undergone clinical assessment and echocardiography between January 2011 to December 2015, and had had a follow-up examination within 1-2 years. Relevant CKCS were identified by review of medical records of all CKCS in the database.

Based on evaluation of MR severity at the first echocardiographic examination, the CKCS were allocated into two groups; a group of CKCS with iMR and an age-matched control group (two controls/case). Age-matching was performed by selecting control dogs from the database with an age as close as possible to dogs with iMR. Exclusion criteria and echocardiographic examination and assessment were identical to that described for substudy 1.
with the exception that no stress test was performed. Selected data from 26 of the dogs are included in nine published studies concerning different research questions (Reimann et al., 2014a, 2014b, 2016, 2017a, 2017b; Cremer et al. 2014, 2015; Meurs et al., 2018; Christiansen et al., 2019).

Statistical analysis

Data were analyzed using statistical software (R studio, version 0.98.1091, 2009-2014 RStudio) and $P<0.05$ was used as the level of significance. Normal distribution of variables within groups was assessed using Shapiro Wilks test and visual inspection of histograms and Q-Q plots. For continuous variables, group differences were investigated using t-tests for variables that followed a normal distribution or Wilcoxon rank sum test for groups that did not. Values reported were means and standard deviations or medians and interquartile ranges as appropriate. Differences in proportions between groups were explored using Fisher's exact tests.

Mean and maximal heart rate response to stress testing were evaluated using linear models for repeated measurements with group (control and iMR), test (stress test 1, stress test 2, and stress test 3), and heart rate at baseline as explanatory variables. An interaction between group and test was included. The individual dog was included as a random variable. In similar models, mean and maximal heart rate changes from baseline were evaluated with only group and test as explanatory variables. For each statistical model, residuals were tested for homogeneity of variance. The models were reduced through backward selection based on $P$ values.
Multinomial logistic regression was used to assess short-term progression of MR. Response variable was disease group at follow-up: iMR, MR<20%; or constant MR, MR≥20%. Disease group at first examination (iMR or control), age at first examination, and follow-up time were included as explanatory variables. Logistic regression analyses were performed in a backward stepwise manner based on $P$ values.

**Results**

*Substudy 1*

In total, 26 CKCS (iMR, $n=13$; controls, $n=13$) were invited for examination. However, nine CKCS (iMR, $n=7$; controls, $n=2$) were excluded because their MR had progressed and they no longer fulfilled the inclusion criteria. In one dog, stress test 3 was not recorded.

The final population included 17 CKCS (iMR, $n=6$; controls, $n=11$) and their characteristics and conventional echocardiographic variables are given in Table 1. Only the LA/Ao ratio differed between the two groups (slightly larger LA/Ao ratio found in dogs with iMR ($P=0.047$)). None of the dogs were receiving medication for cardiovascular disease but one control dog was treated with clomipramine hydrochloride. All control dogs were staged ACVIM A and all dogs with iMR were staged ACVIM B1.

In Table 2, the mean and maximal heart rates recorded during baseline and stress recordings are given. The only variable significantly influencing mean ($P=0.004$) and maximal ($P=0.003$) heart rates in the repeated measures models was heart rate at baseline. Thus, mean and maximal heart rate did not differ significantly between the two groups. No variables
significantly influenced mean or maximal heart rate changes from baseline. Fig. 1 illustrates mean and maximal heart rates for each dog in both groups at baseline 1 and during all stress tests.

Table 3 shows the effect of each stress test on MR. Stress testing did not change MR group for any of the control dogs. During stress testing, iMR was still apparent in 33% (stress test 1) or 50% (stress tests 2 and 3) of the dogs. The severity of iMR changed to a MR<20% in 50% of dogs during all stress tests. During stress test 1, iMR changed to a constant MR≥20% in 1/6 dogs with iMR. This dog still had variable MR sizes (≥20%) but no longer fulfilled the iMR criteria (the majority of MRs were estimated to be 20%). It was not the same dogs in the iMR group that had iMRs during the three stress tests. In Fig. 2, the number of iMR recorded during baselines and stress tests are plotted against mean heart rate; iMR was only recorded at mean heart rates below 150 beats/min (bpm). Supplementary material includes echocardiographic video clips of a dog with intermittent mitral regurgitation at baseline and during the stress tests (Supplementary data files 1-4).

Substudy 2

Fourteen CKCS with iMR and 28 control CKCS were identified. Among these, seven CKCS were also included in substudy 1. Baseline characteristics and conventional echocardiographic variables are presented in Table 4. Only the ejection fraction (EF; 4-chamber view) differed between the two groups and only at the follow-up examination (EF was higher in control dogs; \( P = 0.0008 \)). None of the dogs received medication for cardiovascular disease but one dog was treated with prednisolone (dog with iMR at the first examination), one dog was
treated with clomipramine hydrochloride (dog with iMR at follow-up), and one dog was treated with firocoxib, tramadol, gabapentin and omeprazole (control dog at follow-up). At exam 1, all control dogs were staged ACVIM A and all dogs with iMR were staged ACVIM B1. At exam 2, 11 control dogs were ACVIM A and 17 were ACVIM B1 while two dogs with iMR were staged ACVIM A and 12 were staged ACVIM B1.

Mean follow-up time (months) was the same for the two groups (control group, 18.3 ±2.9; iMR group, 19.0 ±3.4; P = 0.5). In Table 5, the progression of MR in both groups is shown. In the iMR group, 57% of the dogs progressed to a constant MR≥20% compared to 54% in the control group. In the control group, iMR was observed in two (7%) dogs at follow-up. Post hoc sample size determination was performed based on these results (Supplementary data file 5).

Multinomial logistic regression analysis did not generate higher odds (odds ratio [OR], 5.2; 95% confidence interval [CI], 0.7-38.2; P = 0.1) of being in the constant MR (MR≥20%) disease group vs. the control group (MR<20%) at the follow-up examination for CKCS with iMR compared to controls, when the model was adjusted for age. The odds were higher (OR, 12.8; 95% CI, 1.2-139.2) for a CKCS with iMR being in the iMR group vs. control group at follow-up, compared to control CKCS (P = 0.04).

There were higher odds with age (OR, 1.9/year; 95% CI, 1.1-3.3) of being in the constant MR group (MR≥20%) than the MR<20% disease group at follow-up, when adjusting
for disease group at baseline ($P = 0.02$). Length of follow-up time did not significantly influence these results.

**Discussion**

The present study shows that iMR disappeared in 50% of the CKCS when heart rate was increased after auditory stimuli or physical activity. However, no iMRs were recorded at mean heart rates above 150bpm. The heart rate response to stress tests were the same in CKCS with and without iMR. There was no difference in the odds of progression to a more severe disease group (constant MR≥20% vs. MR<20%) in the short-term between dogs with iMR and control dogs. However, increasing age was associated with higher odds of being in a more severe disease group (constant MR≥20% vs. MR<20%) at follow-up, when adjusting for disease group at baseline. Generally, confidence intervals were large and sample sizes low (Supplementary data file 5).

The first part of the study included a stress test. Three different stress tests including both physical activity and sound were chosen to ensure that they would affect all dogs including dogs with different degrees of hearing disability or familiarity with the chosen Stress test sounds. The intention of the selected stress tests was to increase the heart rate of the dogs, although, it must be kept in mind that not only the heart rate will be affected by such testing. Most likely, the stress tests will activate a more generalized neuroendocrine response. The CKCS in the two groups demonstrated a similar heart rate response to the Stress testing. Thus, the current study indicated no difference in autonomic regulation in CKCS with iMR compared to control CKCS.
Interestingly, during stress testing, the MR appeared to be reduced in 50% of the dogs with iMR (they changed to constant MR<20%). There may be several reasons for this observation. One explanation may be that the iMR may have disappeared due to less R-R interval variation (less pronounced SA and/or APC) caused by the increased heart rate (Reimann et al., 2014b).

Change of MR direction may be another explanation for reduction of iMR in response to an increased heart rate. If increased heart rate caused the MR to change direction the entire MR may not be recorded and therefore appear smaller on color Doppler flow echocardiography (Zoghbi et al., 2017). A different explanation could be that in some dogs the iMR may appear only rarely (1-2 min between them) and thus, it is possible that by chance no iMR was recorded. Either way, the findings of this study have clinical importance. When performing an echocardiographic examination, it is important to bear in mind the possible effect of an increased heart rate. In CKCS with increased heart rates (especially if they are above 150 bpm), it may be of value, to assess the MR more thoroughly and wait for a normal heart rate (preferably below 150 bpm, if possible). Likewise, these findings should be kept in mind when auscultating dogs with increased heart rates. Intermittent heart murmurs are possible to auscultate (Beardow and Buchanan, 1993; Häggström et al., 1995), but may not be possible to identify in dogs with increased heart rates. In essence, iMR may easily be overlooked in stressed CKCS, which may be especially important in relation to breeding scheme examinations when screening young CKCS for breeding purposes.
The last part of the study assessed short-term progression (1-2 years) of iMR. We were not able to demonstrate higher odds of progression to a more severe disease group (constant MR≥20% vs. MR<20%) within 1-2 years among CKCS with iMR compared to control CKCS. This is in contrast to a recent study indicating a higher risk of cardiac death later in life in CKCS with iMR at an early age compared to CKCS with MR<20% (Reimann et al., 2017b). Hence, it is possible that a longer period of time or larger sample size would have increased the difference between the two groups.

At the follow-up examination the control group had a significantly higher EF (4-chamber view) compared to the iMR group. In addition, EF (4-chamber view) increased significantly between first and second examinations in the control group, but none of the other echocardiographic variables differed significantly between examinations within the same group (data not shown). The increase in EF may be a result of disease progression given that 1-2 years had passed and many dogs developed a constant MR≥20%. However, the difference in EF (4-chamber) at follow-up between the iMR and control group may also be a random finding (due to multiple testing) and remains unexplained.

Study limitations include a relatively small sample size, which prevented some statistical analyses and may have caused the lack of statistical significance for increased odds of progression to a more severe disease stage among dogs with iMR in the short-term. Furthermore, some dogs may have been misclassified as control dogs because it cannot be ruled out that some ‘control’ dogs did have iMR that was not present on the recordings available. Stress testing may also have influenced the ability to adequately identify iMR because it did
agitate some dogs which made a few echocardiographic recordings suboptimal. In these recordings evaluation of MR was challenging and iMR could have been overlooked. Finally, the use of color Doppler flow echocardiography with known limitations was used for the MR evaluation in this study (Zoghbi et al., 2017).

Conclusions

In 50% of the CKCS with iMR, stress tests reduced the presence of iMR and no iMR was recorded at mean heart rates above 150 bpm. Furthermore, the increase in heart rate as a response to stress tests was similar for CKCS with and without iMR. This study did not demonstrate higher odds for CKCS with iMR compared to controls for progression to a more severe disease stage (constant MR≥20% vs. MR<20%). It is important not to overlook iMR in stressed CKCS with early disease; the importance of this finding is yet to be fully understood.

Conflict of interest

None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper.

Acknowledgements

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of Veterinary and Animal Sciences, University of Copenhagen, Denmark for their technical assistance.
References


Table 1

Dog characteristics and echocardiographic variables of 17 Cavalier King Charles spaniels that underwent stress testing. \(^a\)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Control (n=11)</th>
<th>iMR (n=6)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (F/M)</td>
<td>17</td>
<td>8/3 (73%/27%)</td>
<td>4/2 (67%/33%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Age (years)</td>
<td>17</td>
<td>5.3 ±2.7</td>
<td>4.7 ±2.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Bodyweight (kg)</td>
<td>17</td>
<td>8.6 ±2.1</td>
<td>8.5 ±1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>LA/Ao</td>
<td>16</td>
<td>1.1 ±0.1 (^b)</td>
<td>1.2 ±0.1</td>
<td>0.047</td>
</tr>
<tr>
<td>LVIDSN</td>
<td>17</td>
<td>0.9 ±0.1</td>
<td>0.8 ±0.1</td>
<td>0.9</td>
</tr>
<tr>
<td>LVIDDN</td>
<td>17</td>
<td>1.5 ±0.2</td>
<td>1.3 ±0.05</td>
<td>0.08</td>
</tr>
<tr>
<td>FS (%)</td>
<td>17</td>
<td>35.9 ±6.4</td>
<td>34.4 ±8.2</td>
<td>0.7</td>
</tr>
<tr>
<td>EF(_{\text{lax}})</td>
<td>17</td>
<td>66.5 ±7.6</td>
<td>67.1 ±4.6</td>
<td>0.9</td>
</tr>
<tr>
<td>EF(_{4\text{CH}})</td>
<td>16</td>
<td>67.6 ±5.7</td>
<td>69.8 ±2.0 (^c)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

F, Female; M, Male; BW, Bodyweight; EF\(_{4\text{CH}}\), Ejection fraction 4-chamber view; EF\(_{\text{lax}}\), Ejection fraction long axis view; FS, Fractional shortening; iMR, Intermittent mitral regurgitation; LA/Ao, Ratio of left atrium to aortic root; LVIDDN, Left ventricular end diastolic diameter normalized for BW; LVIDSN, Left ventricular end systolic diameter normalized for BW; NA, Not possible to measure.

\(^a\) Values reported are means ± standard deviation.

\(^b\) Group with reduced number of observations (n=10)

\(^c\) Group with reduced number of observations (n=5)
**Table 2**

Mean and maximal heart rates in 17 Cavalier King Charles spaniels that underwent stress testing.<sup>a</sup>

<table>
<thead>
<tr>
<th></th>
<th>Control (n=11)</th>
<th>iMR (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline Mean</td>
<td>120±25</td>
<td>114±21</td>
</tr>
<tr>
<td>Baseline Max</td>
<td>125±26</td>
<td>121±23</td>
</tr>
<tr>
<td>Stress 1 Mean</td>
<td>148±29</td>
<td>122±25</td>
</tr>
<tr>
<td>Stress 1 Max</td>
<td>171±30</td>
<td>150±44</td>
</tr>
<tr>
<td>Stress 2 Mean</td>
<td>147±31</td>
<td>131±36</td>
</tr>
<tr>
<td>Stress 2 Max</td>
<td>167±39</td>
<td>147±46</td>
</tr>
<tr>
<td>Stress 3 Mean</td>
<td>145±14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>143±32</td>
</tr>
<tr>
<td>Stress 3 Max</td>
<td>154±15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>155±32</td>
</tr>
</tbody>
</table>

iMR, intermittent mitral regurgitation; Max, Maximum.

<sup>a</sup>Values reported are means ± standard deviation.

<sup>b</sup>Group with reduced number of observations (n=10)
Table 3
Mitral regurgitation assessment for each of the 17 Cavalier King Charles spaniels during three stress tests

<table>
<thead>
<tr>
<th>Stress 1</th>
<th>Stress 2</th>
<th>Stress 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>iMR&lt;20%</td>
<td>Constant MR&lt;20%</td>
<td>Constant MR&lt;20%</td>
</tr>
<tr>
<td>iMR 1</td>
<td>(33%)</td>
<td>3</td>
</tr>
<tr>
<td>Constant MR&lt;20%</td>
<td>(50%)</td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>11</td>
</tr>
</tbody>
</table>

n=6; n=11

iMR, Intermittent mitral regurgitation; MR, Mitral regurgitation.

a iMR was defined as MR<20% in the majority of cardiac systoles, but with some MR jets ≥20%.

b Constant MR was defined as constant MR≥20%.

c Control was defined as MR<20%. In one dog stress test 3 was not recorded.
Table 4

Dog characteristics and echocardiographic variables at first examination (exam 1) and follow-up (exam 2) of 42 Cavalier King Charles spaniels.

<table>
<thead>
<tr>
<th></th>
<th>Exam 1</th>
<th></th>
<th></th>
<th>Exam 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (F/M)</td>
<td>iMR</td>
<td>P</td>
<td>Control (F/M)</td>
<td>iMR</td>
<td>P</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>16/12 (57%/43%)</td>
<td>10/4 (71%/29%)</td>
<td>0.5</td>
<td>16/12 (57%/43%)</td>
<td>10/4 (71%/29%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Age (years)</td>
<td>4.6 ±1.9</td>
<td>4.0 ±2.3</td>
<td>0.4</td>
<td>6.1 ±1.9</td>
<td>5.6 ±2.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Bodyweight (kg)</td>
<td>8.7 ±1.4</td>
<td>8.4 ±1.2</td>
<td>0.5</td>
<td>8.9 ±1.5 b</td>
<td>8.7 ±1.2</td>
<td>0.5</td>
</tr>
<tr>
<td>LA/Ao</td>
<td>1.2 ±0.11 c</td>
<td>1.2 ±0.1</td>
<td>0.7</td>
<td>1.2 ±0.1</td>
<td>1.2 ±0.1 d</td>
<td>0.9</td>
</tr>
<tr>
<td>LVIDSN</td>
<td>0.9 ±0.1</td>
<td>0.9 ±0.1</td>
<td>0.8</td>
<td>0.9 [0.8;1.0] b</td>
<td>1.0 ±0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>LVIDDN</td>
<td>1.5 ±0.1</td>
<td>1.5 ±0.2</td>
<td>0.7</td>
<td>1.5 ±0.2 b</td>
<td>1.5 ±0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>FS (%)</td>
<td>32.9 ±5.4</td>
<td>31.7 ±7.6</td>
<td>0.6</td>
<td>35.0 ±7.4</td>
<td>30.7 ±5.1</td>
<td>0.06</td>
</tr>
<tr>
<td>EF_{lax}</td>
<td>68.6 ±5.3 e</td>
<td>65.4 ±4.2</td>
<td>0.06</td>
<td>67.9 ±6.5</td>
<td>64.8 ±5.6</td>
<td>0.1</td>
</tr>
<tr>
<td>EF_{4CH}</td>
<td>65.8 ±6.1 b</td>
<td>63.6 ±4.1</td>
<td>0.2</td>
<td>68.8 ±4.9 e</td>
<td>63.2 ±4.2</td>
<td>0.0008</td>
</tr>
</tbody>
</table>

F, Female; M, Male; BW, Bodyweight; EF_{4CH}, Ejection fraction 4-chamber view; EF_{lax}, Ejection fraction long axis view; FS, Fractional shortening; iMR, Intermittent mitral regurgitation; LA/Ao, Ratio of left atrium to aortic root; LVIDDN, Left ventricular end diastolic diameter normalized for BW; LVIDSN, Left ventricular end systolic diameter normalized for BW.

a Values reported are means ± standard deviation, or median ± interquartiles, as appropriate.

b Group with reduced number of observations (n=27)

c Group with reduced number of observations (n=25)

d Group with reduced number of observations (n=13)

e Group with reduced number of observations (n=26)
Table 5
Progression within 1-2 years of mitral regurgitation in 42 Cavalier King Charles spaniels with intermittent mitral regurgitation or controls.

<table>
<thead>
<tr>
<th>Follow-up examination</th>
<th>iMR a (n=14)</th>
<th>MR&lt;20%</th>
<th>Constant MR b</th>
</tr>
</thead>
<tbody>
<tr>
<td>iMR a (n=14)</td>
<td>4 (29%)</td>
<td>2 (14%)</td>
<td>8 (57%)</td>
</tr>
<tr>
<td>Control c (n=28)</td>
<td>2 (7%)</td>
<td>11 (39%)</td>
<td>15 (54%)</td>
</tr>
</tbody>
</table>

iMR, Intermittent mitral regurgitation; MR, mitral regurgitation.

a iMR was defined as MR<20% in the majority of cardiac systoles, but with some MR jets 
≥20%
b Constant MR was defined as constant MR≥20%
c Control was defined as MR<20%.
Figure legends

**Mean heart rate**

- **Y-axis:** Beats/min
- **X-axis:** B1, S1, S2, S3

**Max heart rate**

- **Y-axis:** Beats/min
- **X-axis:** B1, S1, S2, S3
Fig. 1. Raw data plot mean and maximal heart rates (HR) at baseline 1 (B1) and during stress test 1 (S1), stress test 2 (S2), and stress test 3 (S3) in control dogs and dogs with intermittent mitral regurgitation (iMR). Black symbols represent control dogs. Red symbols represent dogs with iMR (n=17).

Fig. 2. Raw data plot showing the number of intermittent mitral regurgitations (iMR) observed at different mean heart rates at baseline (left) and during stress test (right) recordings in each of the 17 Cavalier King Charles spaniels. Note. There are three observations per dog per figure (baseline 3 and stress test 3 was not recorded in one dog, respectively). Red symbols represent dogs with iMR, black symbols represent control dogs.