# Predictive value of three-dimensional echocardiographic variables on development of myxomatous mitral valve disease in dogs

*Prediktivt värde av tredimensionella ekokardiografiska variabler för utvecklingen av myxomatös klaffsjukdom*

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Swedish University of Agricultural Sciences, SLU
Faculty of Veterinary Medicine and Animal Science
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## Abstract

Myxomatous mitral valve disease (MMVD) is the most common acquired cardiovascular disease and the most common cause of congestive heart failure (CHF) in dogs. Cavalier King Charles Spaniels (CKCS) is one of the breeds most commonly affected by MMVD and disease progression has been shown to occur at a younger age among CKCS, when compared to other breeds. The age of onset of MMVD is an inherited trait among CKCS and the geometry of the mitral valve has recently become of interest among veterinary researchers to obtain deeper understanding of the currently partly understood pathogenesis. Findings suggest that dogs with MMVD and many healthy CKCS share an altered three-dimensional (3D) geometry of the mitral valve annulus, which differs from healthy dogs of other investigated breeds. In this comparison, the mitral valve annulus has been shown to lack the normal saddle-shaped appearance, instead it had a flattened appearance among many of the healthy CKCS and dogs with MMVD. This flattening has been proposed as a factor contributing to the early onset of disease progression in CKCS.

The object of this thesis was to evaluate whether a flattening of the mitral valve (MV) in healthy dogs might contribute to an accelerated progression of MMVD. It was hypothesized that MV variables indicative of flattening, when measured with real-time 3D transthoracic echocardiography (RT3D-TTE), might be used as a prognostic test to determine dogs at risk for progression of early onset or severe MMVD.

The study was conducted as a follow-up of dogs with preexisting RT3D-TTE datasets, obtained 6 years earlier during an initial study that aimed to describe morphological mitral valve variations between healthy CKCS and non-CKCS. The present study included a follow-up questionnaire, distributed to and returned by owners of dogs with preexisting RT3D-TTE datasets, designed to evaluate cause of death (cardiac/non-cardiac) and possible progression to congestive heart failure (CHF) for deceased dogs. The questionnaire also facilitated staging of disease severity among dogs still alive, and owners of alive dogs were invited for a follow-up examination; including auscultation and new standard echocardiographic examination, as well as new RT3D-TTE examination. The following RT3D-TTE MV variables; annulus height (AnH), normalized tenting volume (nTnV), tenting area (TnA) and tenting height (TnH) - proposed to be representative of a flattened appearance of the MV annulus - were measured in datasets from the initial study and compared to the dogs' disease stage at follow-up.

Information was received from a total of 24 dogs (n=18 CKCS and n=6 non-CKCS) from the initial cohort of 29 healthy dogs, 62.5% of these dogs were reported by owners as still alive at time of follow up. Deceased dogs made up 37.5% of the studied population (n=6 CKCS and n=3 non-CKCS). None of the owners of deceased dogs reported cardiac-related deaths, and only one of the deceased CKCS was reported by the owner as having progressed to CHF pre-mortem. The mean age at follow-up for the CKCS (alive and deceased) was 8.6 years [SD 0.43] and among all the CKCS, 56% had developed a heart murmur discovered by a veterinarian at a mean age of 7.8 years [SD 2.6]. Three dogs; n=2 CKCS and n=1 non-CKCS, had progressed to stage B2, i.e. MMVD and signs of volume overload. These dogs had presented with a pronounced flattening of the mitral valve annulus at initial study, as they aggregated at the lower numerical range regarding all four variables.

In conclusion, this particular cohort of dogs were represented by mainly preclinical cases of MMVD, with the exception of one CKCS that had progressed to CHF pre-mortem. Three dogs that had presented with a flattened mitral valve annulus at the initial study, tended to have progressed to MMVD with echocardiographic signs of left sided volume overload at follow-up.



*Keywords:* Cavalier King Charles Spaniel, myxomatous mitral valve disease, three-dimensional echocardiography, prognosis

## Sammanfattning

Myxomatös klaffsjukdom (MMVD) är den vanligaste förvärvade hjärtsjukdomen samt den vanligaste orsaken till kongestiv hjärtsvikt (CHF) hos hund. Cavalier king charles spaniel (CKCS) är en av de raser som vanligtvis drabbas av MMVD och sjukdomsutveckling sker vid en tidigare ålder än hos andra raser. Debutåldern av MMVD är ärftligt hos CKCS och mitralisklaffens geometri har på senaste tiden vunnit intresse hos veterinära forskare i sökandet efter ökad förståelse kring den i nuläget ofullständigt förstådda patogenesen bakom MMVD. Resultat från dessa studier föreslår att hundar med MMVD och många friska CKCS delar en avvikande tredimensionell (3D) arkitektur på mitralisklaffens annulus, som skiljer sig i jämförelser med friska hundar av andra raser som undersökts. I denna jämförelse visades att mitralisklaffens annulus saknade den normala sadellika formen, istället hade annuluset ett mer tillplattat utseende hos många friska CKCS och hundar med MMVD. Detta har föreslagits som en bidragande faktor till den tidiga sjukdomsutvecklingen hos CKCS.

Målet med denna studie var att utvärdera om en tillplattning av mitralisklaffen (MV) hos en grupp friska hundar kan ha bidragit till en tidigare utveckling av MMVD. Hypotesen var att MV-variabler som indikerar tillplattning, mätta med realtids tredimensionellt transthorakalt hjärtultraljud (RT3D-TTE), kan användas som ett prognostiskt test för att upptäcka vilka hundar som riskerar att utveckla tidig eller allvarlig MMVD. Studien var utformad som en uppföljning av hundar som hade undersökts med RT3D-TTE 6 år tidigare, i en studie som syftade till att beskriva morfologiska mitralisklaffsskillnader mellan friska CKCS och icke-CKCS. Uppföljningen bestod av ett frågeformulär, som distribuerades till och fylldes i av ägare, till hundar som hade deltagit i den tidigare studien. Formuläret var utformat för att undersöka dödsorsak (hjärtorsak/annan orsak) samt eventuell progression till CHF hos avlidna hundar. Hundägarnas formulärsvar möjliggjorde kategorisering enligt sjukdomsallvarlighet för hundar som fortfarande levde. Ägarna till de levande hundarna erbjöds även uppföljande undersökning av hundarna; med auskultation, hjärtultraljud och RT3D-TTE. Följande RT3D-TTE MV-variabler; annulus höjd (AnH), normaliserad tältningvolym (nTnV), tältningsarea (TnA) och tältningshöjd (TnH) – har föreslagits representera ett tillplattat utseende hos MV annuluset. Variablerna mättes i RT3D-TTE-bilder från den initiala studien och jämfördes mot hundarnas allvarlighetsgrad av MMVD vid uppföljning.

Information samlades in från totalt 24 av hundarna (n=18 CKCS och n=6 icke-CKCS) ur den initiala studiepopulationen av 29 friska hundar, 62,5 % av dessa hundar rapporterades av ägarna som fortfarande i livet vid uppföljningen. De avlidna hundarna utgjorde 37,5% av studiepopulationen (n=6 CKCS och n=3 icke-CKCS). Ingen av de avlidna hundarna uppgavs av ägaren ha dött av hjärtorsak och endast en av de avlidna CKCS uppgavs av ägaren ha utvecklat CHF före sin död. Medelåldern vid uppföljning för samtliga CKCS inkluderade (levande och avlidna) var 8,6 år [SD 0,43], och bland alla CKCS hade 56 % utvecklat ett blåsljud som upptäckts av veterinär vid en medelålder av 7,8 år [SD 2,6]. Tre hundar; n=2 CKCS och n=1 icke-CKCS, hade fortskridit till sjukdomsstadium B2, dvs. MMVD med tecken på volymöverfyllnad. Vid den initiala studien hade dessa hundar ett tillplattat mitralisannulus, då de fördelades i det lägre spannet på samtliga fyra variabler.

Slutsatserna var att studiepopulationen huvudsakligen utgjordes av hundar med preklinisk MMVD, med undantaget för en CKCS som utvecklat CHF innan sin död. Tre hundar med tillplattat mitralisannulus vid den initiala studien, tenderade att ha utvecklat MMVD med ekokardiografiska tecken på vänstersidig volymöverfyllnad vid uppföljningen.



*Nyckelord:* Cavalier king charles spaniel, myxomatös klaffsjukdom, tredimensionellt hjärtultraljud, prognos

# Table of content

# Abbreviations

| | |
|---|---|
| 2D | two-dimensional |
| 3D | three-dimensional |
| ACVIM | American College of Veterinary Internal Medicine |
| AnH | annulus height |
| Ao | aortic root |
| CHF | congestive heart failure |
| CKCS | Cavalier King Charles Spaniel |
| CPE | cardiogenic pulmonary edema |
| E-peak | peak velocity of early mitral inflow |
| LA | left atrium |
| LV | left ventricle |
| LVIDDn | normalized left ventricular internal diameter in end diastole |
| LVIDSn | normalized left ventricular internal diameter in end systole |
| MMVD | myxomatous mitral valve disease |
| MR | mitral regurgitation |
| MV | mitral valve |
| MVP | mitral valve prolapse |
| nTnV | normalized tenting volume |
| RT3D-TTE | real-time three-dimensional transthoracic echocardiographic examination |
| SLU | Swedish University of Agricultural Sciences |
| TnA | tenting area |
| TnH | tenting height |

# 1. Introduction

Myxomatous mitral valve disease (MMVD) is the most common acquired cardiovascular disease and cause of congestive heart failure (CHF) in dogs. The disease is complex, as it includes structural and hemodynamical changes, as well as biochemical and genetical aspects. Medical management of the disease, aimed at decreasing morbidity and prolonging life span for affected dogs, are successful in many cases, but despite decades of dedicated research, the pathogenesis and etiology of the disease is still not completely understood. There are prognostic tools available to help assess patients at risk of CHF, but a comprehensive and unison understanding as to which dogs develop severe MMVD and why is still to be determined.

The Cavalier King Charles Spaniel (CKCS) is one of the breeds most commonly affected by MMVD, with up to 90% of dogs developing the disease with rising age. The pathogenesis of MMVD among CKCS is divergent to other breeds, in that the disease development occurs at a younger age. Heritability has been established, but the specific underlying genetic mechanisms are yet to be determined. Recent studies have found that the structure of the mitral valve annulus differs in dogs with MMVD when compared to healthy dogs, where the annulus has a more flattened shape in dogs with MMVD. The same flattening of the annulus was found among a group of healthy CKCS when comparing them to a group of healthy dogs of other breeds. A potential connection between the altered structure and progression of disease has to date not been studied.

The aim of this thesis was to evaluate whether a flattened structure of the mitral valve annulus in a group of healthy dogs might contribute to an accelerated progression of MMVD. The hypothesis was that variables indicative of a flattened mitral valve might be used as a prognostic sign to determine dogs at risk for progression of early onset or severe MMVD.

*Figure 1. Cavalier King Charles Spaniel in the color Blenheim. Photo by Marianne Jansson, re-used with permission from dog owner Susanne Stuart.*

# 2. Literature review

## 2.1. Myxomatous mitral valve disease

MMVD is the most common acquired cardiovascular disease and the most common cause of CHF in dogs. Due to the fact that the disease is progressive and its presence is influenced by age, sex and breed, the results from epidemiological studies vary with regards to prevalence (Borgarelli & Buchanan 2012). A fairly recent study conducted in UK produced a prevalence estimate of 3.54% for MMVD amongst a general population of dogs older than 1 year (Mattin *et al.* 2015), while post-mortem prevalence varies between 34.4-69.7% (Das & Tashjian 1965; Whitney 1974; Jones & Zook 1965 see Mattin *et al.* 2015). The breed with the highest risk factor associated with MMVD is CKCS (Mattin *et al.* 2015), but in general geriatric and small to medium sized breeds (<20 kg) are the ones commonly affected (Madsen *et al.* 2011; Aupperle & Disatian 2012; Mattin *et al.* 2015).

CKCS is one of the top three breeds regarding mortality due to cardiac disease and male dogs are reported to have a higher cardiac mortality than females (Egenvall *et al.* 2006). However, many dogs with MMVD remain without clinical signs of disease, some for a comparably long preclinical period and some throughout life. The CKCS dogs have early age of onset of MMVD, which, at a given age, leads to a higher prevalence compared to other breeds (Fox 2012), and the prevalence exceeds 90% in dogs older than 10 years (Borgarelli & Häggström 2010).

Myxomatous changes most commonly appear in the mitral valve, but the other heart valves can also be affected. Even though MMVD is most frequently reported among smaller dogs, large breeds can also be affected but with a different disease progression and a more reserved prognosis (Borgarelli & Häggström 2010).

### 2.1.1. The mitral valve complex

*Macroscopic features of the mitral valve*

The mitral valve consists of four components, all collaborating as a complex throughout the cardiac cycle to optimize central and peripheral circulation. These

components are the mitral valve leaflets, chordae tendineae, mitral annulus and left ventricular papillary muscles (Fox 2012).

In healthy dogs, the annulus is elliptical, and the three-dimensional (3D) structure of the annulus and the leaflets together form a saddle-shape (Menciotti *et al.* 2017) or a hyperbolic paraboloid. This structure plays an important role in reducing the stress on the leaflets and for maintaining valve integrity to avoid regurgitation during systole (Fox 2012; Menciotti *et al.* 2018).

The two mitral valve leaflets are thin, translucent structures that separate the left atrium from the left ventricle during systole. The anterior/septal leaflet is larger and longer than the posterior/lateral leaflet but the histological components are the same (Fox 2012).

*Microscopic features of the mitral valve*

Both leaflets consist of four distinct layers. The *fibrosa* is a core of compact collagen that connects the leaflets to adjacent components in both aspects (Markby *et al.* 2017). It is covered by the *spongiosa*, a layer of connective tissue mainly made up of proteoglycans and glycosaminoglycans. *Atrialis* is the surface layer of the leaflet, aimed at the left atrium, consisting mainly of endothelium (Borgarelli & Buchanan 2012), fibers and in the proximal third of the leaflet also smooth muscle cells (Fenoglio *et al.* 1972). The *ventricularis* is on the opposite side of the leaflet, facing the left ventricle, and is histologically similar to the atrialis but lacks smooth muscle cells.

In the distal aspect of the leaflet, the fibrosa continues and constitutes the core of the chordae tendineae, which is covered in endothelium. The chordae tendineae are various sized fibrous strings and connect the distal end of the leaflets to the left ventricular wall via the papillary muscles (Fox 2012). At the chordae-papillary junction the dense collagen bundles of the chordae divide into multiple processes and intertwine with the cardiac muscle bundles of the papillary. This is considered the weakest point of the entire mitral complex (Fenoglio *et al.* 1972).

In the proximal aspect of the leaflet, the fibrosa continues and connects to the mitral annulus, which consists of collagen, elastin and cartilage in various thickness. The annulus outlines the orifice of the mitral valve complex and functions as a hinge in the leaflet-atrium and leaflet-ventricle junctions. It also collaborates with the fibrous skeleton of the heart to reinforce the myocardium and prevent excessive dilatation of the orifice (Fox 2012).

### 2.1.2. Myxomatous degeneration of the mitral valve

*Etiology & pathogenesis*

The exact etiology of MMVD is unknown and disease mechanisms remain to be determined, but much research has been devoted to better understand the patho-

genesis and etiology of the disease. Due to the many different aspects of the disease, this thesis does not account for all of them, but focuses specifically on the structural changes that occur in the mitral valve complex, left ventricle (LV) and left atrium (LA).

Due to the high prevalence within the breed, it has been proposed and proven that MMVD is inheritable among CKCS (Swenson *et al.* 1996). Several interesting loci have been identified and are suspected to play a role in the inheritance (Madsen *et al.* 2011; Bionda *et al.* 2020), but studies have yet to produce reproducible results as to which loci are responsible (O'Brien *et al.* 2021). An association between parent and offspring regarding presence and severity of MMVD have been described in CKCS, suggesting that the disease is polygenically inherited (Swenson *et al.* 1996; Madsen *et al.* 2011), but the exact genetic mechanisms remain to be fully understood (Fox 2012; O'Brien *et al.* 2021).

Whole genome sequencing has recently shed some light on the inheritance of MMVD. The genome of 8 common dog breeds, one of which was the CKCS, has been sequenced and studied. It was found that the CKCS genome carried an increased number of harmful genetic variations, when compared to the other 7 investigated breeds. When compared to Dachshunds, the deviation in the genome of the CKCS was found to be correlated to the regulation of a certain myocardial protein, NEBL, which was found to be down-regulated in papillary muscles in diseased dogs. This finding is interesting because papillary muscles are essential for maintaining integrity of the mitral valve annulus, and hence this genetic variant prevalent among CKCS could provide an explanation to the early onset and high prevalence of MMVD in the breed (Axelsson *et al.* 2021). However, there are not yet any genetic tests available.

Genetics is thought to be a major determinant, which in combination with one or several factors might contribute to an increased risk for development of disease among predisposed dogs. Loss of or damage to the endothelial lining of the mitral valve leaflets is believed to play a role in the pathogenesis, as it might contribute to disturbances in the communication with subendothelial cells. Valvular interstitial cells (VICs) are a type of subendothelial cells that might be changed and activated upon lack of communication with endothelial cells. Serotonin has also been proposed as a factor contributing to the activation of VICs (Ljungvall & Häggström 2017).

### *Macroscopic features of myxomatous changes to the mitral valve complex*

Elongation of chordae tendineae with subsequent bulging of associated sections of the mitral valve leaflet edges, resulting in prolapse of the valve towards the left atrium (LA), might be considered an early sign of MMVD. In human medicine this phenomenon is referred to as mitral valve prolapse (MVP) (Häggström *et al.* 2004; Borgarelli & Häggström 2010). Movement of the leaflets into the LA can also be

caused by rupture of degenerated chordae tendineae, causing the mitral valve to 'flail' into the atrium.

Nodular lesions on the mitral valve leaflets due to myxomatous degeneration is the most common gross pathological sign of MMVD, however the grade and significance of changes varies a lot (Fox 2012). The myxomatous degeneration is often more pronounced on the anterior/septal mitral leaflet, but both leaflets can be affected (Menciotti *et al.* 2017). To grade the gross leaflet pathology, a classification scale according to Whitney is often used. The severity of lesions has been shown to correlate with advancing age. Grade 0 refers to normal leaflets, whereas grade 1 refers to the appearance of discrete nodules on the free edge of the leaflets and might be difficult to differ from a normal leaflet. Grade 2 leaflets display larger nodules, whereas progression to grade 3 involves the nodules merging. Grade 4 refers to the most advanced stages of the disease, where the leaflets are grossly distorted and accompanied by thickening and possibly rupture of the chordae tendineae (Fox 2012; Markby *et al.* 2017).

*Microscopic features of myxomatous changes to the mitral valve complex*

Histologically, the myxomatous degeneration and enlargement occurs due to an increased concentration of glycosaminoglycans in the spongiosa layer of the leaflets (Häggström *et al.* 2004; Borgarelli & Buchanan 2012), accompanied by destruction or loss of the fibrosa (Markby *et al.* 2017). Disruption of the collagen distribution and degradation rate in the spongiosa has also been proposed to play a role in the pathogenesis (Keene *et al.* 2019), as well as proteolytic enzymes called matrix metalloproteinases (MMPs). MMPs could be involved in the disorganization of the connective tissue in spongiosa (Ljungvall & Häggström 2017). Electron microscope imaging of diseased leaflets have been used to describe areas that lack endothelium altogether, revealing the underlying extracellular matrix (Corcoran *et al.* 2004).

*Pathophysiological changes secondary to mitral valve degeneration*

The progression of the disease involves diffuse enlargement of the valve leaflets and further deformation of the valve apparatus, resulting in mitral regurgitation (MR) (Fox 2012). Primary MR occurs due to the myxomatous degeneration of the leaflets, resulting in a backward flow of blood into the LA during systole. The volume of the MR is considered the major determining factor to the degree of left sided dilatation, as the LA and LV dilates in response to the increased volume to protect the pulmonary vessels from venous congestion and maintain forward cardiac output (CO). This contributes to a decreased resistance (afterload) for the LV, whose function is to pump blood forward into the rest of the body, due to loss of stroke volume into the LA. To compensate this loss of stroke volume, the LV increases its end-diastolic-volume (EDV). The decreased afterload in combination

with the increased burden of volume and filling pressure, as the MR from the dilated LA returns to the LV in diastole, contributes to an increased preload for the LV. In response to the increased preload, the myocardium of the LV undergoes pathological growth, referred to as eccentric hypertrophy. Depending on the severity of the MR, this may result in normal or hyperdynamic contractility of the LV, referred to as hyperkinesia. In summary - the compensatory actions of the LV result in dilatation with maintained wall thickness, to normalize pressure and maintain forward CO, with or without ventricular hyperkinesia. The dilatation of the LA and LV results in further distortion of the mitral valve annulus, thus contributing to more regurgitation over the valve, referred to as secondary MR.

As long as the progression of disease occurs slowly, the LA has time to adapt to the increased volume by dilatation. Meanwhile compensatory remodeling of the LV contributes to maintained myocardial systolic function. However, with increasing pathological hypertrophy, the LV eventually loses its contractility and hence ability to compensate and maintain systolic function (Ljungvall & Häggström 2017).

*Three-dimensional structure of the mitral valve in dogs with myxomatous disease*

A recent study has shown that the 3D-structure of mitral valves in healthy CKCS are different than those in healthy dogs of other breeds. In CKCS, the annulus has been shown to be larger and more circular than the normal elliptical shape. In dogs of this breed, the saddle-shaped appearance of the annulus is less pronounced because it is flattened compared to annuli of dogs of other breeds (Menciotti *et al.* 2017). The deviant structure may alter the dynamics of the valve and might increase the stress acting on the leaflets and the chordae. This change in geometry might stimulate development and progression of MMVD lesions and, thereby, increasing regurgitation over the valve (Fox 2012). Flattening of the mitral valve annulus has been proposed as a factor contributing to the early progression of the disease among CKCS, but to date there have been no longitudinal studies to test this hypothesis (Menciotti *et al.* 2018).

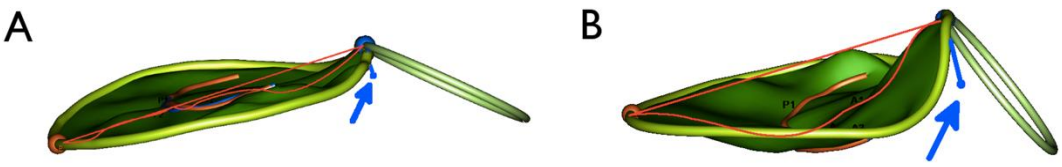


*Figure 2. Mitral valve model obtained using RT-3DTTE and offline analysis of a Cavalier King Charles Spaniel (left, A) and a dog of another breed (i.e., Basenji) (right, B). It can be noticed that the leaflet tenting (space comprised by the red line) and the height of the annulus (blue bar pointed by the blue arrow) are reduced in the Cavalier King Charles Spaniel. From: Comparison of the mitral valve morphologies of Cavalier King Charles Spaniels and dogs of other breeds using 3D transthoracic echocardiography, Journal of Veterinary Internal Medicine (Menciotti et al. 2018:1568). Re-used with permission from John Wiley and Sons.*

### 2.1.3. Diagnosis

The clinical manifestation of MMVD ranges from mild lesions and no signs of disease with a comparably good long-term prognosis and long expected survival time, to severe MMVD lesions with severe regurgitation and, if not already present, an impending risk of CHF and cardiac death/euthanasia (Borgarelli *et al.* 2008; Borgarelli & Häggström 2010; Fox 2012).

*Auscultation*

A common clinical finding in dogs with MMVD is the presence of a systolic murmur over the mitral valve area. In more advanced cases, where cardiac enlargement is present, the murmur might radiate to other ostia. If the tricuspid valve is also affected, a murmur might be detected over the tricuspid area as well (Borgarelli & Häggström 2010). The intensity of the mitral murmur has been shown to be associated with severity of MMVD in small breed dogs (Ljungvall *et al.* 2014), why the presence of a mitral murmur at auscultation in a dog with otherwise typical characteristics should lead a clinician to suspect MMVD. However, it is important to perform an echocardiographic examination to exclude other cardiac pathologies as the source of the murmur and to confirm the diagnosis (Häggström *et al.* 2004).

*Echocardiography*

To date, the diagnosis of MMVD is commonly made using standard echocardiography, why this section is intended to introduce the reader to commonly used terminology and facilitate better understanding of the following sections of this document. There are several imaging modalities that can be used in clinical practice to assess the anatomy and the function of the heart, a brief introduction to the three main modalities are presented below.

Two-dimensional (2D) echocardiography is commonly used to visualize the anatomy of the chambers, valves and vessels, to perform linear measurements and to evaluate the size and systolic function of the ventricles. M-mode provides a one-dimensional view of the movement of the cardiac walls and chambers and can be used to measure cardiac wall thicknesses and chamber diameters, which is useful for assessment of cardiac systolic function. Doppler echocardiography can be used to evaluate blood flow pattern over valves and to assess velocity and volume of blood flow. There are three types of Doppler modalities available, and usefulness of each modality depends on the characteristics of the blood flow to be assessed. Pulsed wave (PW) Doppler echocardiography is useful to study normal non-turbulent flow of comparably low velocity, while color flow (CF) and continuous wave (CW) Doppler echocardiography can be used to detect regurgitation over insufficient valves. Depending on blood flow velocity, PW and CW can both be used for estimation of pressure gradient between cardiac chambers and for indirect

quantification of blood flow. However, CW Doppler provides information about the velocity of the flow even at high velocities.

There are two main trans-thoracic acoustic windows that can be used to visualize the heart with ultrasonography in dogs, the right and left parasternal windows. The right parasternal window is located in the intercostal spaces of ribs 3-6, on the ventral aspect of the right thorax and is accessed with the patient in right lateral recumbency. The left parasternal window is located in the intercostal spaces of ribs 3-4 (cranial) or 5-7 (caudal/apical) close to the sternum on the left side of the thorax and is accessed with the patient in left lateral recumbency. The goals of the echocardiographic examination is to detect and quantify the primary cardiac lesion, determine the etiology and possible presence of coexisting abnormalities, as well as determine and assess the size and function of all four chambers (Pariaut 2011).

*Evaluation of diseased valves*

The first step towards a diagnosis of MMVD is to assess the structure of the mitral valve leaflets and evaluate the presence of MR. Identification of MR can be done using Doppler, either CF or CW (Häggström *et al.* 2004). When examining the leaflets it is important to assess the entire mitral valve, as pathological changes may vary in severity and distribution between individuals. To assess the occurrence of MVP in dogs, a recommendation is to use a minimum of two views (Borgarelli & Häggström 2010). There might be abnormal mitral valve morphology without leakage and there might be MVP without MR, why it is important that the echo-cardiography is performed by a trained operator (Häggström *et al.* 2004).

The 3D morphology of the mitral valve has been subject to evaluation using a modality called real-time three-dimensional transthoracic echocardiography (RT3D-TTE). The results have been used to describe several structural differences within and among groups of dogs. Significant differences were found between healthy CKCS and healthy dogs of other breeds regarding the annulus height (AnH), tenting height (TnH), tenting area (TnA) and normalized tenting volume (nTnV). The reduced AnH, TnH, TnA, nTnV among the healthy CKCS is proposed to represent a flattened shape of the mitral valve apparatus (Menciotti *et al.* 2018). Similar alterations, of the same four variables, were found when comparing various breeds of dogs with MMVD but without cardiac enlargement to healthy dogs (Menciotti *et al.* 2017). These differences are proposed by the authors as potential factors that might predispose development of MMVD among CKCS, but this hypothesis needs to be tested (Menciotti *et al.* 2018).

*Evaluation of changes secondary to valve disease*

After confirming the diagnosis of MMVD, the next step is to determine presence and severity of hemodynamic changes secondary to the structural valve disease. This is done with a number of measurements, designed to evaluate three main areas

of interest: evaluation of MR, level of cardiac enlargement and approximation of filling pressure in the LV (Vezzosi *et al.* 2021).

*Mitral regurgitation*

When it comes to quantification of MR, there are several methods described and used in clinical practice. One method proposed is to visualize the MR-jet using CF Doppler, see Figure 3, estimate the area of the LA and then subjectively compare the area covered by the jet with the total area of the LA (Pariaut 2011). However, this method is semiquantitative and might vary with settings on the ultrasound machine. Other methods, such as measurement of the width of vena contracta, which is the narrowest part of the regurgitant jet immediately distal to the mitral valve, and the proximal isovelocity surface area (PISA) have been described in dogs with MMVD. Some of these methods might be considered time consuming and limited by many assumptions and multiple measurements, which limits their utility in the routine echocardiographic examination (Ljungvall & Häggström 2017).

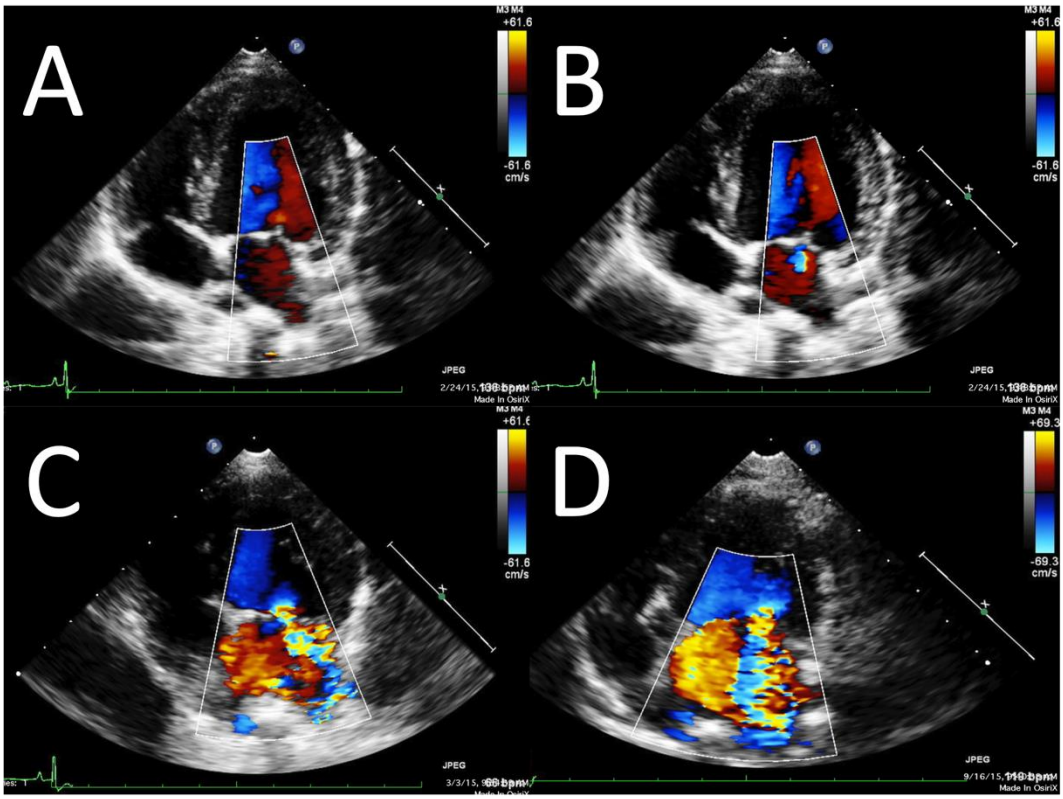


*Figure 3. Pictures showing different degrees of mitral regurgitation (MR) during systole, visualized with Doppler modality in left-sided four-chamber view. Normal mitral valve and no MR (A), mild myxomatous mitral valve disease (MMVD) and MR (B), moderate MMVD and MR (C), severe MMVD and MR (D). Pictures by Jens Häggström, re-used with permission from the originator.*

The severity of MR has also been subject to recent RT3D-TTE studies, with regards to association between traditionally obtained 2D-measurements and volumetric measurements. A strength with the RT3D-TTE system, when compared to traditional 2D-measurements, is that it provides an image of the regurgitant jet that accounts for the actual shape. It does not rely on estimates of a predetermined shape as is the case with 2D-methods. However, since the RT3D-TTE system is not yet in wide clinical use there are no global echocardiographic criterion in place. Furthermore, survival analysis of volumes obtained with RT3D-TTE, and hence their potential use as prognostic indicators of disease severity, are yet to be published (Müller *et al.* 2017; Tidholm *et al.* 2017).

*Enlargement of left atrium and left ventricle*

To determine the presence of and quantify the degree of LA enlargement, a well described and proven method is the left atrium to aortic root-ratio (LA:Ao). Linear diameter measurements of the left atrium and the aortic root can be obtained in right parasternal short-axis view at the aortic level in early diastole (Hansson *et al.* 2002). A ratio between the two that exceeds 1.6 is widely considered indicative of enlargement of the left atrium and, because of the indexation to the aorta, the method is not body size dependent (Misbach *et al.* 2014; Keene *et al.* 2019).

To assess the systolic function of the LV, the left ventricle internal diameter in end diastole (LVIDd) and end systole (LVIDs) can be obtained in right parasternal short-axis M-mode (Pariaut 2011). An increased LVIDs is indicative of a decreased systolic function (Borgarelli *et al.* 2007; Häggström *et al.* 2008). When normalized for body weight, LVIDd can be used as an indication of LV enlargement. A LVIDDn $\geq$ 1.7 (Cornell *et al.* 2004) has been decided severe enough to warrant medical treatment (Keene *et al.* 2019). However, evaluation of systolic function in dogs with MMVD can be complicated, as the compensatory actions of the LV wall might result in hyperkinesia (Ljungvall & Häggström 2017).

Because the LA and LV are 3D structures, they can expand in multiple directions upon dilatation and hence make it difficult to correctly capture the dilatation with traditional 2D-echocardiography. Commonly used methods to estimate LA and LV volume based on measurements obtained with 2D echocardiography have been tested against RT3D-TTE measurements to examine their agreement. Some of the tested 2D-methods had a good agreement, while others did not (Tidholm *et al.* 2011; Tidholm *et al.* 2019).

When evaluating LV enlargement with RT3D-TTE, an association was found between severity of disease and level of volume expansion. In severe cases of MMVD, the greatest expansion occurred in the mid-segment of the LV, resulting in a rounding of the apical and basal parts of the LV. Monitoring this alteration of shape and volume in affected dogs could be helpful in assessment of disease

severity, as it could be used to distinguish dogs at risk for development of severe MMVD (Ljungvall *et al.* 2011).

*Approximation of filling pressure*

Velocities of the mitral inflow into the left ventricle during diastole can provide estimates about the filling pressure in the LV. Early ventricular filling, referred to as the E-wave, represents the first phase of the mitral inflow. The peak velocity of the E-wave (E-peak) can be measured using CW Doppler and used as an indication of increased filling pressure if elevated (Morgan *et al.* 2020).

### 2.1.4. Progression of disease

The American College of Veterinary Internal Medicine (ACVIM) published a consensus statement in 2019, with guidelines for diagnosis and treatment of MMVD. This includes detailed criteria for the different disease stages and hence the level of morbidity, where an increased morbidity correlates with the presence of clinical signs associated with CHF. The different stages are described in Table 1. The echocardiographic criteria are considered the most reliable way to distinguish between stages B1 and B2 (Keene *et al.* 2019).

*Table 1. American College of Veterinary Internal Medicine (ACVIM) consensus stages of myxomatous mitral valve disease (MMVD). Congestive heart failure (CHF), left atrium to aortic root ratio (LA:Ao) normalized left ventricle internal diameter in early diastole (LVIDDn), mitral regurgitation (MR), mitral valve prolapse (MVP).*

| ACVIM-stage | Characteristics | Echocardiographic findings | Medical treatment |
|---|---|---|---|
| **Stage A** (Healthy) | Dog at risk for development of MMVD | None | None |
| **Stage B1** (Preclinical) | Dog with MMVD, but without clinical signs of CHF | MR<br>Structural changes to mitral valve leaflet/s<br>+/- MVP<br>LA:Ao <1.6<br>LVIDDn <1.7 | None |
| **Stage B2** (Preclinical) | | MR<br>Structural changes to mitral valve leaflet/s<br>+/- MVP<br>LA:Ao ≥1.6<br>LVIDDn ≥1.7 | Pimobendan |

| | | | |
|---|---|---|---|
| **Stage C** (Clinical) | Dog with past or current signs of CHF, due to MMVD | | Pimobendan, loop diuretics, potentially other cardiac medications |
| **Stage D** (Clinical) | Dog with CHF due to severe MMVD, demanding intensive medical treatment | | Pimobendan, loop diuretics, other cardiac medications |

*Preclinical disease*

The level of valvular insufficiency and volume overload is in direct relation with the morbidity in dogs with MMVD (Fox 2012). To decrease or delay morbidity, the goal of therapeutic interventions is to counteract the hemodynamic effects of the volume overload to increase survival time in dogs with preclinical or clinical disease (Boswood *et al.* 2016; Keene *et al.* 2019). Initiating treatment for preclinical cases, at the time when cardiac enlargement is severe enough, leads to a reduced heart size. This considerably delays progression of disease to CHF and thus increases life span for affected dogs (Häggström *et al.* 2013; Boswood *et al.* 2018). Mild to moderate cases of MMVD (stages A-B) do not present with clinical signs related to the heart condition (Ljungvall & Häggström 2017).

*Clinical signs of congestive heart failure*

Many dogs with MMVD develop CHF with time, as the mechanisms contributing to congestion include MR and subsequent increase in filling pressures of the LV. These dogs present with clinical signs associated with cardiogenic pulmonary edema (CPE) and/or effusions (pericardial, pleural, ascites) (Fox 2014). CPE occurs due to an increased hydrostatic pressure in the pulmonary capillaries and veins resulting in an increased amount of interstitial fluid. The clinical presentation depends on the amount of fluid and hence the degree of respiratory distress (Sjaastad *et al.* 2010), why it varies from an increased respiratory rate (RR) to dyspnea. Signs of decompensated CHF often arise due to left-sided congestion, why presentation of clinical signs often include anxiousness at night, sternal positioning and abnormal breathing. If the congestion is right-sided, the clinical presentation is more likely to be episodes of syncope, intolerance to exercise and/or weakness (Ljungvall & Häggström 2017).

The presence of cough in dogs with MMVD poses a significant risk factor for progression of disease to CHF (Borgarelli *et al.* 2012). However, cough cannot be considered a specific marker for cardiac disease and should warrant further investi-

gation (Ljungvall & Häggström 2017). Primary tracheobronchial disease is common in older small breed dogs, why it is important to determine if the cough arises from CPE or not (Borgarelli & Häggström 2010). It has been suggested that the lung congestion that occurs due to CHF does not cause coughing, but that there is either abnormal radiographic airway findings or mechanical pressure from LA enlargement that contribute to the cough in patients with MMVD (Ferasin *et al.* 2013). Dilatation of the LA and increased pressure in the pulmonary veins can be considered one of the main pathophysiological mechanisms contributing to clinical signs in dogs with CHF, why respiratory signs might arise due to either CPE, mechanical compression of airways or most likely a combination of the two (Ljungvall & Häggström 2017).

*Diagnosis of congestive heart failure*

Standard clinical approach to detect CPE in dogs include abnormal sounds upon thoracic auscultation and the manifestation of interstitial or mixed interstitial-alveolar lung patterns upon thoracic radiography (Hori *et al.* 2020). Prominent breathing sounds and the presence of abnormal sounds is often best heard at end-inspiration upon auscultation (Ljungvall & Häggström 2017).

Pericardial lung ultrasonography has been proposed as a useful method in the detection of CPE, where the identification of an artifact referred to as B-lines can be associated with CPE in dogs with MMVD. B-lines are formed upon fluid accumulation in the pulmonary parenchyma and present as discrete vertical hyperechoic lines (Hori *et al.* 2020). However, the use of ultrasonography in the evaluation of the thoracic cavity might not always be ideal, as it might not allow complete visualization of the pulmonary parenchyma.

Thoracic radiographs may remain useful in that aspect, as it may provide a view of the entire thorax (Pariaut 2011). Vertebral heart score (VHS) is a way to determine the presence of cardiomegaly in thoracic images, however due to differences in configuration of the thorax between breeds it is important to use breed adjusted measurements (Keene *et al.* 2019). Signs of right-sided CHF, such as pleural effusion, can often be visualized on thoracic radiographs. CPE can possibly be detected on thoracic radiographs, the likelihood increases with severity of MMVD. However, agreement between degree of cardiomegaly and severity of CPE might be considered poor. The optimal way to detect CHF in a dog with MMVD is considered monitoring the resting respiratory rate (RRR) and sleeping respiratory rate (SRR), which should be <30 breaths/minute whilst asleep or at rest in a home environment. An increased RRR and/or SRR might be indicative of emerging or existing CHF and should warrant further investigation and confirmation (Ljungvall & Häggström 2017).

*Treatment of congestive heart failure*

Therapeutic interventions to relieve patients of respiratory distress caused by CPE are aimed at reducing pre- and afterload. Common practice is the administration of a loop diuretic, such as furosemide, that induces rapid diuresis and natriuresis (Fox 2014). In dogs presenting with clinical signs of CPE, established to be subsequent to MMVD, treatment with pimobendan should be started. If the patient is already undergoing treatment, it should be continued (Häggström *et al.* 2013; Keene *et al.* 2019). The mechanism of action for pimobendan is positive inotropy and vasodilatation (Ramsey 2011). Other cardiac medications are also frequently used as supplement.

### 2.1.5. Prognostic variables

Even in dogs with moderate to severe CHF due to MMVD, survival time can be relatively long with medical treatment (Borgarelli & Häggström 2010). There is evidence that certain variables can be used as prognostic tools to estimate survival and predict progression of disease to onset of CHF (Keene *et al.* 2019).

*Auscultation*

Characteristics of the murmur can be used as a tool to assess severity of disease and hence facilitate better understanding of the morbidity and risk factors for individual dogs. The intensity of the murmur has been shown to be associated with the severity of MR (Häggström *et al.* 1995; Ljungvall *et al.* 2009). Furthermore, a murmur with a high intensity is associated with an increased risk of CHF and pulmonary hypertension. In this study, the presence of a loud murmur implied that the dog was more likely to progress to CHF, whereas none of the dogs with mild murmurs presented with CHF. The author proposes that this association has clinical implication, in that a dog with suitable characteristics (small breed, appropriate age) presenting with a mild mitral murmur is likely to be a preclinical case of MMVD (Ljungvall *et al.* 2014).

*Clinical signs*

An increased heart rate (HR), RR and RRR has been shown to be indicative of CHF (Boswood *et al.* 2020). An age exceeding 8 years, the occurrence of syncope, HR >140/min, dyspnea and treatment with furosemide are all associated with an increased risk of cardiac death (Borgarelli *et al.* 2008).

*Echocardiographic variables*

Progressive enlargement of LA/LV is considered moderately predictive for the time aspect of disease progression to CHF (Keene *et al.* 2019). Furthermore, echocardio-

graphic signs of LA/LV enlargement positively correlates to ACVIM stages (Franchini *et al.* 2021).

Enlargement of the LA, linearly measured using the previously described LA:Ao, is one of the echocardiographic dimensions that has been studied in correlation to survival. A ratio exceeding 1.7 has been shown to be associated with an increased risk of cardiac death (Borgarelli *et al.* 2008; Baron Toaldo *et al.* 2018). Even a LA:Ao exceeding 1.4 has been proven significant, proposing that also mild LA enlargement constitutes a risk factor associated with cardiac death (Borgarelli *et al.* 2012). The results are reproducible even in larger cohorts of dogs with MMVD, suggesting that monitoring the LA:Ao is a reliable and well proven method to determine dogs at risk for progression of disease to CHF and cardiac related death (Häggström *et al.* 2008; Boswood *et al.* 2018).

Volume estimates could potentially be used to provide prognostic information, as a maximum volume of the LA exceeding 3.53 ml/kg was shown to be a predictor of cardiac death (Baron Toaldo *et al.* 2018). Normalized LVIDs (LVIDSn) has been shown to have a prognostic value. An increased LVIDSn was found to be indicative of increased risk for cardiac death later in life, when studied in a group of young CKCS (<3 years) (Reimann *et al.* 2017). The increased risk for cardiac death or worsening of cardiac disease was also presented in a group of dogs with CHF due to MMVD, suggesting that increased LVIDs might be prognostic for all stages of disease (Häggström *et al.* 2008). When studied in a group of dogs with preclinical MMVD, it was found that a decreased LVIDDn in response to medical treatment, was associated with an increased time to onset of CHF or cardiac death (Boswood *et al.* 2018).

*Doppler findings*

The characteristics of the transmitral flow is a useful independent prognostic variable, as several studies have shown an association between elevated peak E-wave velocity (>1.2 m/s) and increased risk of cardiac death (Borgarelli *et al.* 2008; Borgarelli *et al.* 2012; Sargent *et al.* 2015). Doppler measurements of the transmitral flow could also be used to calculate mitral E/A ratio. Atrial contraction, referred to as the A-wave, represents the last phase of the mitral inflow. An E/A ratio >2 could pose a negative prognostic sign, as it has been shown to be associated with increased risk for cardiac death (Borgarelli *et al.* 2012).

Moderate to severe MR, when approximated with CF Doppler as the systolic regurgitant area in relation to the area of the LA, could be considered a negative prognostic variable. Even if intermittent, MR of said severity has been shown to be associated with cardiac death later in life, when studied in a group of young CKCS (< 3 years) (Reimann *et al.* 2017).

*Biomarkers*

NT-proBNP is a peptide measurable in plasma, released in response to stretching of the atria and/or ventricles, increased concentrations of which can be used as a biomarker indicating increased cardiac filling pressures. The prognostic value of NT-proBNP has been evaluated, and the results suggested that decreased concentration after treatment of CHF could be associated with better outcome for dogs with MMVD (Hezzell 2019).

Troponin is a myofibril protein measurable in plasma. Increased circulating concentrations of troponin have been shown to correlate to severity of myocardial injury in response to several etiologies. When studied in dogs with MMVD, an increased concentration was found to be associated with advancing severity of disease. However, it was discussed that troponin might be considered a suboptimal biomarker, when used by itself, to determine severity of MMVD. It was however suggested useful when used in combination with other biomarkers (Ljungvall 2011).

*Prognostic models*

The MINE score is a recently published echocardiographic scoring system, designed to help in the assessment of disease severity and prognosis. It consists of four echocardiographic variables, LA:Ao, LVIDDn, fractional shortening (FS) and E-peak, all of which are independent predictors of cardiac death (Vezzosi *et al.* 2021). FS can be calculated from measurements of LVIDs and LVIDd, and is a variable used to assess the systolic function of the heart (Pariaut 2011). When combined and implemented in dogs with preclinical MMVD, these variables have been suggested to define dogs at risk for progression to CHF or cardiac death (Vezzosi *et al.* 2021).

The recently published DELAY study produced a model that combines the use of biomarkers with echocardiographic variables. An increased concentration of NT-proBNP was shown to correlate with progression to CHF. When adjusted for LA:Ao and E-peak, the model was suggested prognostic in predicting cardiac death and progression of disease for dogs with MMVD (Borgarelli *et al.* 2021).

In summary, there are several variables that can be used as prognostic markers for morbidity and mortality. However, the use of these variables is limited to distinguishing among patients with ascertained MMVD, i.e. stage B1 and B2, and identifying individuals at risk of CHF, as they change when CHF is imminent or present (Häggström *et al.* 2009). Further studies are needed to provide information about which stage A individuals are at risk for future development of severe MMVD.

# 3. Material and methods

A cohort of 29 dogs, 21 CKCS and 8 non-CKCS, were contacted via e-mail or phone by the author for a follow-up. The dogs had previously been enrolled in a study evaluating mitral valve morphology using RT3D-TTE (Menciotti *et al.* 2018) and thus had RT3D-TTE datasets eligible for measurements. The owners of dogs still alive were offered reexamination at SLU in Uppsala.

This study was approved by the Ethical Committee for Animal Welfare at SLU in Uppsala, SLU-ID C12/15 and 5.8.18-04682/2020.

## 3.1. Study design

*Initial study*

In the study by Menciotti *et al.* 2018, dogs had been prospectively enrolled following interview with the owner, physical examination; including comprehensive cardiac auscultation, conventional 2D and Doppler echocardiography and acquisition of a RT3D-TTE dataset. Datasets were obtained throughout the year of 2015.

To be enrolled in the study, the dogs had to be older than 1 year of age and without a history of cardiac disease. Additional inclusion criteria were tolerance of a complete echocardiographic examination without need of sedation, absence of evidence of MMVD or other cardiac disease at 2D and Doppler examinations, and acquisition of RT3D-TTE datasets free from significant stitching artifacts. Dogs with murmurs graded 1/6 or 2/6 were not excluded provided that all other inclusion criteria were met.

*Follow-up*

All of the dogs enrolled in the initial study were contacted for the follow-up, carried out approximately 6 years after the initial study, and the ones that accepted participation were all included. Medical treatment directed at CHF was not a criterium for exclusion, but the medication and doses were taken into record and used for categorizing deceased dogs according to disease severity.

Owners reported outcome for the dogs via a questionnaire (see Appendix 1) or a standardized phone interview based on the questionnaire, which was designed to evaluate general health, potential occurrence of clinical signs associated with CHF

and causes of death for deceased dogs (Freeman *et al.* 2005; Häggström *et al.* 2013; Boswood *et al.* 2018).

The follow-up examination included physical examination, cardiac auscultation and acquisition of complete 2D/Doppler and RT3D-TTE datasets by JH or IL at SLU in Uppsala.

## 3.2. Data management

Data describing sex, age, BW and breed was entered into a spreadsheet for all dogs included, along with MV variables from previous RT3D-TTE datasets. For deceased dogs, date and cause of death (cardiac/non-cardiac) and occurrence of CHF was recorded. For dogs still alive, variables from the 2D-echocardiographic follow-up were used to categorize and verify the disease status as unchanged (A) or of increased severity (B1, B2, C), when compared to initial examination.

For the alive dogs that were not available for follow-up echocardiographic examination, the disease status was also entered as unchanged or of increased severity, but based on the report from the owner. Because verification of disease stage could not be performed with echocardiographic examination, dogs were considered stage A/B1 regardless of whether the owner reported that the dog had a murmur or not, given that the dog was not currently treated with pimobendan. Dogs were considered stage B2 if the owner reported that the dog had a murmur and was currently treated with pimobendan.

## 3.3. Statistical analysis

Statistical analysis was performed with the help of JH using commercially available software (JMP Pro v16.0, Cary, NC, USA). To investigate if dogs having progressed to stage B2 differed from those in stages A/B1 at follow-up, regarding the RT3D-TTE obtained mitral valve variables that were significantly different between CKCS and non-CKCS dogs in the initial study, variables and disease stage were plotted together and compared visually.

To determine survival time for deceased dogs, age from birth to reported date of death was calculated and converted into time in years. For deceased dogs where the exact date of death had not been reported by the owner, date of death was standardized to the median time within the reported year or period of death.

To determine age at onset of murmur, dates were obtained from the database for Swedish CKCS, to which the occurrence of a consistent systolic murmur detected by a veterinarian is reported. In dogs where murmur had debuted at unknown time during a period between two known dates, the date for onset of murmur was set as median time within this period.

# 4. Results

Out of the 29 dog owners contacted, 24 (83%) accepted participation in follow-up and 5 dogs (17%) were lost to follow-up or declined further participation by the owner. Out of the 24 dogs, n=18 were CKCS and n=6 were non-CKCS. The non-CKCS dogs included Basenji, Volpino Italiano, Border Collie, Afghan, German Terrier and Mixed Breed (n=1 of each) dogs.

At time for follow-up, n=15 (62.5%) of the dogs were still alive, and n=12 of the alive dogs were CKCS and n=3 were non-CKCS. Out of the alive dogs, n=8 (53%); n=7 CKCS and n=1 non-CKCS, underwent physical and echocardiographic examinations. The outcomes for these dogs are presented in Table 2. None of the dogs alive had progressed to CHF.

*Table 2. Outcome for dogs alive at follow-up (n=15). Comparison of disease stage of myxomatous mitral valve disease (MMVD) according to American College of Veterinary Internal Medicine (ACVIM) guidelines between initial exam and follow-up. At initial exam, all dogs included were considered stage A. Follow-up echocardiographic examinations and auscultation were performed by JH or IL, dogs examined and auscultated are described as verified. For dogs that did not undergo echocardiographic examination or auscultation, the stage of MMVD was based on questionnaire and/or phone interview answers by the owners. These dogs are described as reported by the owner and the non-CKCS dog that had progressed to stage B2 was of the breed Volpino Italiano.*

| | **Unchanged (Stage A)** | **Stage A/B1** | **Stage B1** | **Stage B2** |
|---|---|---|---|---|
| **Verified (n=8)** | **2** | **-** | **6** | **-** |
| CKCS | 1 | - | 6 | - |
| non-CKCS | 1 | - | - | - |
| **Reported by owner (n=7)** | **1** | **4** | **-** | **2** |
| CKCS | - | 4 | - | 1 |
| non-CKCS | 1 | - | - | 1 |

At follow-up, n=9 (37.5%) of the dogs were deceased, n=6 of these were CKCS and n=3 were non-CKCS. Cause of death and age at death for deceased dogs are presented in Table 3. One (n=1) of the deceased CKCS had signs of CHF pre-mortem, reported by the owner. None of the non-CKCS dogs had progressed to CHF.

*Table 3. Cause of and age at death for all deceased dogs (n=9), Cavalier King Charles Spaniels (CKCS) and dogs of other breeds.*

| Cause of death | Number of CKCS | Age at death (years) |
|---|---|---|
| Trauma (n=2) | n=1 | 7.6 and 9.1 |
| Joint disease (n=2) | n=2 | 5.2 and 6.2 |
| Unknown (n=2) | n=1 | 5.9 and 10.7 |
| Stroke (n=1) | n=1 | 12.6 |
| Chronic kidney disease (n=1) | - | 9.9 |
| Pyometra (n=1) | n=1 | 7.7 |

A total of 10 (56%) out of all CKCS included in the study had a murmur detected and reported by a veterinarian. Mean age at onset of murmur was 7.8 years [SD 2.6]. Result of comparison between disease stage at follow-up for all dogs included, to the RT3D-TTE obtained mitral valve variables at initial study are presented in Figure 4.

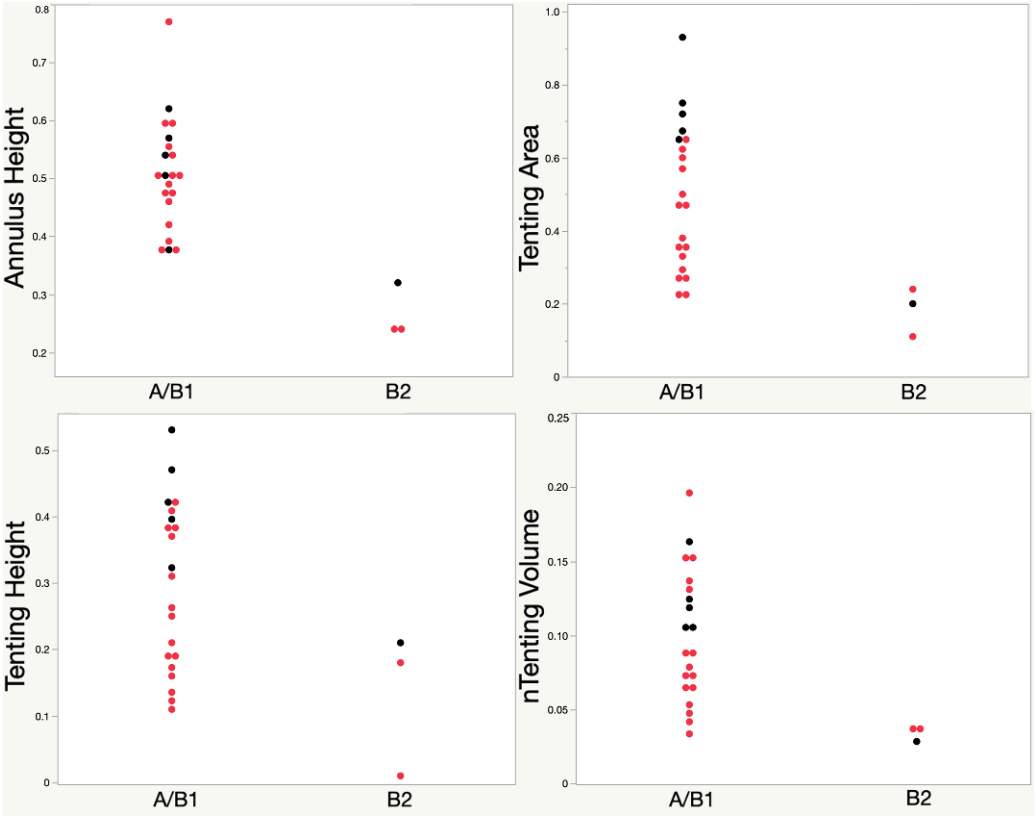


*Figure 4. Plot of mitral valve variables (y-axis), obtained with real-time three-dimensional transthoracic echocardiography (RT3D-TTE) at initial study, by American College of Veterinary Internal Medicine (ACVIM) disease stage at last follow-up (x-axis) for all included dogs (n=24). Examined RT3D-TTE variables included were those that were found significantly different between Cavalier King Charles Spaniels (CKCS) and non-CKCS dogs, and included annular and tenting height (AnH, TnH), tenting area (TnA) and body-weight normalized tenting volume (nTnV). CKCS in red, dogs of other breeds in black. Stages A/B1 presented together to highlight potential differences from the more advanced stages, i.e. stage B2.*

Among the CKCS, n=15 (83%) had been used for breeding; n=3 were male and n=12 were female. Mean number of offspring from these dogs was 13.5 [SD 13.3]. Regarding occurrence of MMVD among close relatives, n=16 (89%) of the CKCS in this study had parents who had developed MMVD. Among the CKCS, n=7 (39%) of the dogs were close relatives, and the relationships between these dogs are presented in Table 4.

*Table 4. Nature of relationships between close relatives (n=7) among the Cavalier King Charles Spaniels.*

| **Relationship** | **Number of dogs** |
|---|---|
| Parent/offspring | n=2 offspring with one common parent |
| Sibling | n=3 and n=2 from different parentage |

The average age at initial exam was 3.2 years [SD 0.39] for CKCS and 5.7 years [SD 0.64] for non-CKCS. The mean age at follow up for CKCS (n=18) was 8.6 years [SD 0.43], and for non-CKCS (n=6) 11.0 years [SD 0.75]. The mean time to follow up was 5.4 years [SD 0.35] and 4.4 years [SD 0.67] for CKCS and non-CKCS respectively.

# 5. Discussion

Three previously healthy dogs with RT3D-TTE mitral valve variables reflecting a flattened shape of the mitral valve annulus, tended to have progressed to stage B2 of MMVD at follow-up. Interestingly, these dogs were not only represented by CKCS, a breed known to be predisposed to MMVD, but also a Volpino Italiano. MMVD is not a disease unique to CKCS, as it is especially common among small breed geriatric dogs. The mean age at follow-up for the non-CKCS dogs in this study was 11 years, which might provide an explanation. The results might still be indicative of an association between mitral valve flattening and progression of MMVD, regardless of breed.

In this study, we did not diagnose many dogs with severe MMVD and CHF, i.e. stages B2-D. This might be due to the limited sample of dogs and may not reflect the general population of stage A dogs. It can be argued that a larger scaled study would have increased the chances of coming across a larger number of severe cases and might have produced a different outcome. It is, furthermore, a positive finding that, with the exception of one dog, the majority had not developed signs of severe MMVD and CHF despite a comparably long follow-up period. However, the mean age at follow-up in this study was relatively low for the CKCS (8.6 years), when compared to results from previously published follow-up studies. In the QUEST-study by Häggström *et al.* (2013), as well as the longitudinal study by Borgarelli *et al.* (2012), the median age at follow up for CKCS was 10 years. Combined with the fact that the mean age at onset of murmur in this particular study was 7.6 years, it can be hypothesized that a longer follow-up period might have resulted in a different outcome with regards to occurrence of severe MMVD. This hypothesis can be supported by results from Häggström (1996), where the prevalence of MMVD exceeded 90% in CKCS older than 10 years.

*Causes of death*

The comparably young age at which some of the CKCS had died is notable. This might be indicative of co-morbidities, worthy of taking into consideration when performing future studies. It can also be suggested to have contributed further to the limitation of the study population, as the possible progression of MMVD among these beforehand deceased dogs could not be reviewed. A remarkable finding among the deceased dogs, was that only one CKCS was reported with signs of CHF

and the rest were reported deceased due to non-cardiac causes. This might be viewed as further indication of the benign nature of MMVD progression among some dogs. It may also be attributed to the type of owners, whose dogs were included in this study. Many of the CKCS in the initial study were provided by breeders, committed to improve cardiac health among the Swedish population of CKCS.

*Heritability & breeding*

Among the CKCS in the study population, n=7 were close relatives and 83% had been used for breeding. Due to the fact that only one of the studied dogs had developed signs of CHF during the follow-up period, it can be concluded that the majority of this particular cohort of dogs had not progressed into severe MMVD at the time of follow-up. The role of heritability in this aspect is of interest, because it might be argued that the genetic lines of the dogs studied may be the result of successful breeding recommendations and responsible breeding. It could also be viewed as representative of the type of owners and dogs provided to the initial study, as previously discussed. However, future studies of prevalence among Swedish CKCS are needed to prove this assumption and provide statistical evidence to support.

The prevalence of MMVD among Swedish CKCS was studied between 1985-1991 (Häggström 1996) and most recently in 2008 (Lundin 2008). In 2008, it was concluded that there had been no decline in prevalence of MMVD among CKCS, when compared to the previous study. In 2017, the breeding recommendations for CKCS in Sweden was revised. The differences, when compared to previous recommendations (SCKCS 2005), was that the age of MMVD debut was increased from 2 to 3 years of age, meaning that dogs have to reach 3 years of age and be auscultated free from murmur before being used for breeding. Another difference was the introduction of a limit, as to how many litters a male dog can contribute to before he reaches 6 years of age. Additionally, the age limit of murmur onset among male dogs was revised from 7 to 8 years of age, meaning that if the male presents with a murmur at the age of 8 years, he can still be used in breeding. Finally, the age requirement for parental dogs was increased from 4 to 5 years, meaning that a potential parent has to reach 5 years of age and be auscultated free from murmur before producing any approved litters (SKK 2016).

Even though these recommendations were not implemented until after all of the CKCS included in this study had already been born, they might be interpreted as the result of prolonging age to onset of MMVD among Swedish CKCS. When taking this into consideration, it might prove difficult to conduct studies aimed at finding the dogs with early onset MMVD, without including a large number of dogs. However, the American dogs also included in the initial study might represent a greater genetic diversity, and thus provide intriguing results upon follow-up.

*Future research*

The geometry of the mitral valve complex remains of interest, as it might contribute to an increased understanding about the etiology and pathogenesis of MMVD. Prognostic variables, to be used on an individual level, might benefit dogs and owners in the long-term management of the disease and hence are worthy of a continued interest by researchers and clinicians. There is a shared motivation among veterinarians, researchers, breeders and owners for clarity, as to which dogs develop severe or early onset MMVD. To achieve this, further collaboration between all parties is needed to reach the common goal - healthy dogs and a complete understanding of all the aspects of MMVD.

*Limitations*

Longitudinal studies provide a relatively strong level of evidence to support a hypothesis, and thus, can be considered a desirable way to conduct research in veterinary medicine. However, this study design comes with a number of challenges, which might make them hard to execute in an optimal manner. The occurrence of the Covid-19 pandemic provided yet another obstacle, to get the dogs to come for the follow-up echocardiographic examination. This resulted in a relatively uncertain and blunt categorization of 47% of the dogs still alive. To base categorization of disease severity on the occurrence of medication might be suboptimal, as it may not be considered strong evidence of severity of MMVD. The assumption is based largely on the preferences and abilities of a number of different attending clinicians - a factor that is impossible to verify and standardize.

The RT3D-TTE system provides many possibilities, both of a clinical nature but also as a tool to be used in research. However, in this particular study, datasets acquired from several dogs in the initial study could not be analyzed owing to so-called stitching artefacts. This limited the final study population, as only datasets free from significant stitching artefacts obtained in the initial study could be used in the statistical analysis.

*Conclusions*

Three dogs with RT3D-TTE mitral valve variables reflecting a flattened shape of the mitral valve annulus at initial study, had a tendency to have progressed to stage B2 of MMVD at follow-up. It can be concluded that the studied cohort of dogs was mainly represented by preclinical cases of MMVD at time of follow-up. Further and larger scaled studies are needed to refute or verify the hypothesis, that dogs with a flattened mitral valve annulus develop early onset MMVD or are at risk of progression to severe MMVD.

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# Popular science summary

*Introduction*

Myxomatous mitral valve disease (MMVD) is the most common acquired heart disease and the most common cause of congestive heart failure in dogs. The disease is complex and involves structural and hemodynamical changes, as well as biochemical and genetical aspects. The progression of disease is often relatively slow and characterized by a long preclinical period, during which the dog does not display any clinical signs of disease. However, MMVD is a chronic condition and affected dogs are at a higher risk of developing congestive heart failure over time. Medical treatment aimed at reducing the consequences of the disease is often successful and can increase survival time with maintained quality of life in affected dogs, even in dogs with moderate to severe heart failure. The Cavalier King Charles Spaniel (CKCS) is one of the breeds most commonly affected, in CKCS disease progression occurs at a younger age than in other breeds and the prevalence among geriatric CKCS (>10 years) can exceed 90%.

*Normal mitral valve complex*

The mitral valve complex consists of several components, all of which are important to maintain and optimize blood flow during each heartbeat. The mitral valve is located on the left side of the heart and separates the atrium from the chamber. The normal architecture of the mitral valve complex forms a saddle-like shape in healthy dogs, with an elliptical annulus functioning as hinges for the leaflets, the thin sheets that prevent the blood from flowing backwards into the atrium as the heart contracts.

*Myxomatous changes to the mitral valve complex*

In dogs with MMVD, changes can occur in one or several of the components of the mitral valve complex, thus compromising the integrity of the structure. The most common gross pathological sign is nodular changes to the mitral valve leaflets, making them lose their thin and sheet-like appearance and enabling the blood to flow backwards over the valve as the heart contracts. This backward flow of blood, referred to as regurgitation, can be heard upon auscultation as a murmur. Over time, this regurgitation causes the heart to change its shape, as the left atrium expands to

withstand the increased blood volume provided by the regurgitation. The left chamber must work harder to maintain blood flow to the body, as well as incorporate the increased volume of blood from the expanded atrium, and therefore compensates by changing its shape. When the changes become too severe, the heart is unable to compensate and heart failure develops.

Recently the three-dimensional geometry of the mitral valve was subject to studies, using a ultrasonographic method called real-time three-dimensional echocardiography (RT3D). Results from these studies described that there was a difference in the architecture of the mitral valve, when comparing dogs with MMVD to healthy dogs of other breeds. The mitral valve annulus, among the dogs with MMVD, was found to have a more flattened appearance than the normal saddle-shape. The same difference in architecture was found in a later study, when comparing healthy CKCS to healthy dogs of other breeds.

*Diagnosis*

The diagnosis of MMVD is traditionally made using two-dimensional ultrasonography of the heart, after the discovery of a murmur over the mitral valve area upon auscultation. The measurements obtained during the 2D-ultrasonography provide an indirect quantification of the disease effects on the heart, and can also provide some information about the prognosis. However, the prognostic variables currently available can only distinguish which individuals, already affected by MMVD, that are at risk for future development of heart failure. They cannot be used to identify which healthy individuals that are at risk for future development of severe disease, as they can only be used after identification of a murmur or ultrasonographic characteristic findings of the disease in affected dogs.

*Objective, material and methods*

The object of this thesis was to evaluate whether a flattening of the mitral valve in healthy dogs might contribute to an accelerated progression of MMVD. The hypothesis was that RT3D variables indicative of a flattened mitral valve, might be used as a prognostic test to detect individuals at risk for severe or early onset MMVD.

This study was conducted as a follow-up study for dogs that had previously been examined with RT3D, both CKCS and dogs of other breeds. The owners were contacted and were asked to complete and return a questionnaire, dogs still alive were offered a follow-up examination; with auscultation as well as new standard ultrasonographic and RT3D examinations of the heart. For deceased dogs, information provided via the questionnaire was used to determine whether the dog had died from cardiac-related or other reasons, and if the dog had displayed signs of heart failure prior to death.

*Results and conclusions*

Three dogs with a flattened appearance of the mitral valve at initial examination, tended to have progressed to MMVD with echocardiographic signs of volume overload, i.e. changes to the shape and size of the left atrium and/or the left chamber. The group of dogs studied was, at time of follow-up, mainly represented by mild cases of MMVD. Additional and larger scaled studies are needed to further investigate the hypothesis, and to provide more understanding of the mechanisms that contribute to early onset MMVD, to help identify individuals at risk for progression to severe MMVD.

# Populärvetenskaplig sammanfattning

*Inledning*

Myxomatös klaffsjukdom (MMVD) är den vanligaste förvärvade hjärtsjukdomen samt den vanligaste orsaken till hjärtsvikt hos hund. Sjukdomen är komplex, med både strukturella förändringar samt förändringar i blodtryck och blodflöde, såväl som biokemiska och genetiska aspekter. Sjukdomsförloppet är ofta relativt långsamt och karaktäriseras av en lång preklinisk period, under vilken hunden inte visar några kliniska tecken på sjukdom. MMVD är en kronisk åkomma, varför drabbade hundar löper en högre risk att drabbas av hjärtsvikt över tid. Medicinsk behandling hjälper till att bromsa konsekvenserna av sjukdomen och många gånger lyckas man med behandling förlänga livslängden med bibehållen livskvalité för drabbade hundar, även hos de med måttlig till allvarlig hjärtsvikt. Cavalier King Charles Spaniel (CKCS) är en ras som ofta drabbas av MMVD. Sjukdomsförloppet skiljer sig hos CKCS, då de ofta utvecklar sjukdom vid tidigare ålder än andra raser. Sjukdomsförekomsten inom rasen är hög, hos individer äldre än 10 år kan upp till 90 % ha utvecklat sjukdom.

*Det normala mitraliskomplexet*

Mitraliskomplexet är den hjärtklaff som skiljer förmak från kammare i vänster hjärthalva. Komplexet består av flertalet komponenter som samarbetar för att upprätthålla och optimera blodflödet vid varje hjärtslag. Hos friska hundar bildar komplexet en sadelformad struktur, med ett elliptiskt annulus. Annuluset fungerar som gångjärn för de tunna segelklaffarna, vars uppgift är att förhindra att blodet flödar baklänges in i förmaket när hjärtat kontraherar.

*Myxomatösa förändringar i mitraliskomplexet*

Hos hundar med MMVD kan en eller flera av komponenterna i mitraliskomplexet vara påverkade, vilket gör att strukturen är förändrad med nedsatt hållfasthet. Den vanligaste förändringen är förtjockning av klaffseglen, vilket medför att de tappar sin förmåga att hålla tätt mellan förmak och kammare. Klafförändringen leder till ett bakåtläckage av blod när hjärtat kontraherar, detta turbulenta blodflöde kallas regurgitation och ger upphov till ett blåsljud som kan höras med stetoskop. Med tiden medför regurgitationen att vänster förmak expanderar, för att inrymma den

ökade blodvolymen som flödar baklänges. Vänster kammare får jobba hårdare för att upprätthålla blodcirkulationen i kroppen, men måste samtidigt inrymma den extra blodvolymen som skickats upp i det expanderade förmaket, och kompenserar genom att förändra sin form. När förändringarna blir så omfattande att hjärtat inte längre lyckas kompensera utvecklas hjärtsvikt.

Mitraliskomplexets tredimensionella arkitektur har nyligen studerats med en ultraljudsteknik som kallas realtids-tredimensionellt hjärtultraljud (RT3D). Det påvisades att det föreligger skillnader i utseendet på mitralisannuluset hos hundar med MMVD, jämfört med normala hundar. Annuluset har ett mer tillplattat utseende samt saknar den sadelliknande formen hos hundar med MMVD. Samma avvikande arkitektur påvisades när man jämförde friska CKCS med friska hundar av andra raser.

*Diagnos*

Diagnosen MMVD ställs vanligtvis efter ultraljudsundersökning av hjärtat hos hund med blåsljud över mitralisområdet. Med ultraljudet kan hjärtats storlek och funktion utvärderas, vilket indirekt ger en bild av sjukdomseffekterna och kan bidra med prognostisk information gällande sjukdomsförloppet hos den enskilda individen. De prognostiska variabler som finns tillgängliga kan i dagsläget användas för att avgöra vilka hundar, med konstaterad MMVD, som riskerar att utveckla hjärtsvikt till följd av sin hjärtsjukdom. Än finns inga variabler tillgängliga som kan avgöra vilka friska hundar som riskerar att utveckla allvarlig sjukdom, innan de utvecklat blåsljud eller förändringar synliga vid hjärtultraljud.

*Mål, material och metod*

Målet med denna studie var att undersöka huruvida en tillplattad form på mitraliskomplexet hos en grupp friska hundar kan ha bidragit till en snabbare utveckling av MMVD. Hypotesen var att RT3D-variabler, som anses indikera en plattare form på mitraliskomplexet, potentiellt kan användas som prognostiskt test för att fånga upp hundar som riskerar att utveckla sjukdom, antingen allvarlig eller vid tidig ålder.

För att undersöka detta gjordes en uppföljning av en grupp hundar, både CKCS och hundar av andra raser, som tidigare genomgått RT3D. Hundägarna kontaktades och fick fylla i ett frågeformulär, de hundar som fortfarande levde erbjöds en uppföljande undersökning; med auskultation av hjärtat såväl som nytt standard-hjärtultraljud och RT3D. För avlidna hundar användes svaren i formulären för att avgöra huruvida de dött av hjärtrelaterade problem eller av annan orsak, samt om de visat tecken på hjärtsvikt innan sin död.

*Resultat och slutsatser*

Tre hundar som hade ett tillplattat mitraliskomplex vid den initiala studien, tenderade att ha utvecklat MMVD med ultraljudstecken på volymöverfyllnad, dvs förändrad form och storlek på vänster förmak och/eller vänster kammare. Den studerade gruppen hundar utgjordes vid uppföljningen huvudsakligen av milda fall av MMVD. Fler och större studier behövs för att undersöka sambandet vidare, samt för att fortsätta utöka förståelsen om mekanismerna som leder till tidigt debuterande MMVD, för att hjälpa till att identifiera individer som riskerar att utveckla allvarlig MMVD.

# Appendix 1

## Follow up questionnaire

**All information regarding you and your dog will be treated confidentially and according to the SLU GDPR-policy.**
https://www.slu.se/om-slu/kontakta-slu/personuppgifter

## Part 1

### General information

**Name of owner:**
**Name of dog:**
**Registered name of dog:**
**Date of birth:**

**Gender**
☐ Female ☐ Male

**Neutered**
☐ Yes ☐ No

**Weight**:
☐ Normal ☐ Underweight ☐ Overweight

**Has your dog been used for breeding?**
☐ Yes ☐ No
**If Yes, how many offspring does the dog have?**

**Have any close relatives to your dog been diagnosed with heart disease?**
*(Parents, siblings and offspring are considered close relatives)*
☐ Yes ☐ No

**Is your dog alive today?**
☐ Yes ☐ No

**If your dog is alive, move on to part 2. If your dog is not alive, move on to part 3.**

# Part 2

## Health

**1. How do you consider the general health of your dog at home?**

*(Pick one option)*

☐ Very bad ☐ Bad ☐ Okay ☐ Good ☐ Excellent

**2. Has your dog been in need of veterinary care over the last 6 years (vaccination not included)?**

☐ Yes ☐ No

If Yes, what was the problem?

## Medication

**3. Is your dog being treated with any medication?**

☐ Yes ☐ No

If Yes, which medication is your dog currently on?

**4. Has there been any dosage changes?**

☐ Yes ☐ No

If Yes, which medicine was changed?

If Yes, to what dose?

If Yes, has the dosage change helped with the dogs' problems?

## Heart specific questions

**5. Do you monitor the breathing rate of your dog during sleep at home?**

☐ Yes ☐ No

**If Yes, which approximate rate does your dog usually have (breaths/minute)?**

☐ less than 10/min ☐ 10-20/min ☐ 20-30/min ☐ 30-40/min ☐ over 40/min

**6. Do you consider the breathing of your dog good at home?**

☐ Yes ☐ No

If No, describe the breathing difficulties:

**7. Does your dog cough?**

☐ Yes ☐ No

**If Yes, when does the dog cough?**

☐ While resting/sleeping ☐ When active ☐ Other, please elaborate:

**If Yes, has there been a change in the frequency of the cough over the last month?**

☐ Increased frequency ☐ Unchanged frequency ☐ Decreased frequency

**If Yes, does the cough interfere with daily activities for your dog?**
☐ Yes ☐ No

**8. Has your dog had any episodes of fainting or collapse?**
☐ Yes ☐ No
If Yes, approximately how many?

**9. How has the level of energy of your dog been over the last month?**
☐ Very low ☐ Below normal ☐ Normal ☐ Above normal ☐ Very energetic

**10. Have you noticed any difficulty for your dog during recreational activities, for example playtime in the yard, playing fetch, walks, other?**
☐ Yes ☐ No
If Yes, describe the difficulty:

**11. Have you noticed any of the following problems in your dog?**
*(Mark the problems that your dog has been displaying, leave empty if you dog has not displayed any of the problems)*
☐ General tiredness
☐ Nighttime anxiety
☐ Decreased appetite
☐ Loss of weight
☐ Increased abdominal circumference
☐ Exercise intolerance

## History

**12. Does your dog have a veterinarian or clinic that it regularly visits?**
☐ Yes ☐ No

**13. If Yes, do we have your approval to access medical records of your dog from the attending clinician, to apprehend necessary clinical history?**
☐ Yes ☐ No

**14. Would you and your dog be interested in a follow up echocardiography?**
☐ Yes ☐ No

# Thank you for your participation!

# Part 3

## Last month alive

**We are sorry for your loss. In this part, we ask you to look back to your dogs' last month alive, to get a better understanding about what was going on during this period.**

**1. When did your dog die?** (Year and month)

**2. Why did your dog die?**

☐ High age
☐ Decreased quality of life
☐ Tumour disease
☐ Kidney disease
☐ Metabolic disease
☐ Trauma or accident
☐ Joint disease
☐ Signs of heart failure (fluid in the lungs/thorax and/or abdomen)
☐ Signs of heart disease not responding to treatment (for example persistent arrythmia/cardiac rhythm disturbance despite directed treatment, fluid in lungs/thorax and/or abdomen not improving with diuretic treatment)
☐ Other cardiac disease than myxomatous mitral valve disease
☐ Unknown
☐ Other

If Other, please elaborate:

**3.How did your dog die?**

☐ Euthanized by veterinarian
☐ Died after a period of illness
☐ Died suddenly
☐ Other

If Other, please elaborate:

**4. How would you describe the general health of your dog during its' last month alive?**

☐ Very poor ☐ Poor ☐ Okay ☐ Good ☐ Excellent

**If you want to describe your dogs' general health during the last month in your own words, you can do it here:**

**5. Did your dog display any of the following problems during its' last month alive?**

*(Pick one or several)*

☐ General tiredness
☐ Nighttime anxiety
☐ Decreased appetite
☐ Loss of weight
☐ Increased abdominal circumference
☐ Exercise intolerance
☐ Cough
☐ Fainting/collapse
☐ Breathing difficulties
☐ None of the above is accurate for my dog

**6. Did you monitor the breathing rate of your dog during sleep at home?**

☐ Yes ☐ No

**If Yes (and if you remember), which approximate rate did your dog usually have (breaths/minute)?**

☐ less than 10/min ☐ 10-20/min ☐ 20-30/min ☐ 30-40/min ☐ over 40/min

**7. Was your diagnosed with myxomatous mitral valve disease before it died?**

☐ Yes ☐ No

**If Yes, did it receive any medical treatment?**

☐ Yes ☐ No

If Yes, which medications did you dog receive?

**8. How was myxomatous mitral valve degeneration diagnosed in your dog?**

☐ Auscultation with stethoscope
☐ Thoracic imaging
☐ Echocardiography
☐ Autopsy
☐ Other

If Other, please elaborate:

**9. Was an autopsy performed on your dog?**

☐ Yes ☐ No

If Yes, what was found:

## History

**10. In the last 6 years, did your dog require veterinary care (besides vaccinations)?**

☐ Yes ☐ No

If Yes, what was the concern:

**11. Did your dog have a veterinarian or clinic that it visited regularly?**

☐ Yes ☐ No

**12. If Yes, do we have your approval to access the medical records of your dog from the attending clinician, to apprehend necessary clinical history?**

☐ Yes ☐ No

# Thank you for your participation!