Evaluation of factors that might affect the grading of heart murmurs in dogs with myxomatous mitral valve disease

Utvärdering av faktorer som kan inverka på graderingen av blåsljud hos hundar med myxomatös mitralisklaffsjukdom

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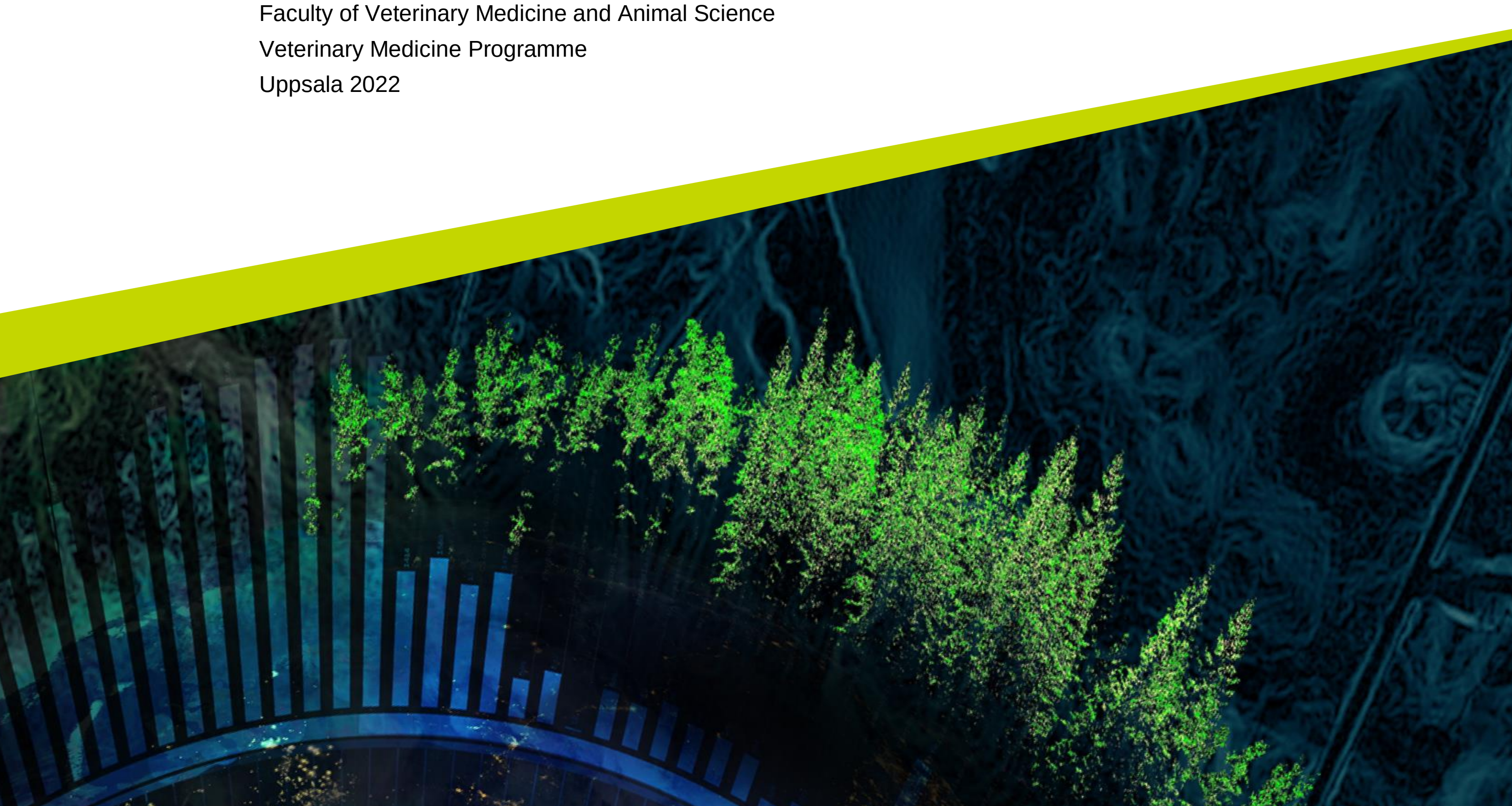

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Abstract

Myxomatous mitral valve disease (MMVD) is the most common heart disease in dogs. The first sign of MMVD is usually a heart murmur, which most commonly is detected before clinical signs of the disease potentially develop. The preclinical period of MMVD is usually long and can be prolonged with medical treatment. Murmur grading and, primarily, diagnostic imaging techniques are used to determine if medical treatment is indicated. Many veterinarians use a 6-level scale to grade heart murmurs. The grades of the 6-level scale are, however, defined differently in various literature. Several studies have shown a relatively good agreement between murmur grade and disease severity based on echocardiographic assessment in dogs with MMVD. However, the agreement is not complete.

The aim of this study was to evaluate potential factors, such as dog and mitral regurgitation (MR) characteristics, that might impact murmur assessment, as well as to evaluate the utility of various murmur grading scales in a group of dogs with MMVD. Evaluation of the utility of various murmur grading scales could potentially help optimize existing murmur grading scales. Moreover, knowledge about factors impacting murmur grading could potentially lead to a better understanding of the association between murmur grades and disease severity (based on echocardiographic assessment).

Client-owned dogs presenting with a heart murmur at the Cardiology Clinic at the University Animal Hospital at the Swedish University of Agricultural Sciences (SLU) in Uppsala, Sweden, were prospectively recruited to the study if they, based on echocardiographic findings, had a diagnosis of MMVD. Information about dog characteristics, data from clinical examination (including murmur characteristics), and echocardiographic findings were collected. The MMVD severity staging system (including stages A, B1, B2, C, and D) developed by the American College of Veterinary Internal Medicine (ACVIM) Specialty of Cardiology Consensus panel was used.

A total of 53 dogs were recruited to the study. A systolic heart murmur could be heard when listening with the ear close to the chest wall (without a stethoscope) in 44%, 86%, and 100% of dogs in stages B1, B2, and C-D, respectively. The murmurs remained audible when the stethoscope was lifted slightly off the chest wall in 11%, 18%, and 63% of dogs in stages B1, B2, and C-D, respectively. None of the dogs in stage B1 had a precordial thrill, whereas 43% and 78% of dogs in stage B2 and stages C-D, respectively, had precordial thrills. The murmur grade increased with ACVIM stage. Most dogs (65% and 83%, respectively) in stages B1 and B2 had an MR jet with a lateral direction, while most dogs (60%) in stages C-D had an MR jet with a central direction.

In conclusion, whether the murmur can be heard without a stethoscope and specific intensity descriptions for each grade might be redundant criteria in murmur grading scales. Both the 4-level and 6-level scales might be useful in dogs with MMVD, but more research is needed to further evaluate the utility of these and other scales. Furthermore, no firm conclusions about if the factors investigated impact the agreement between murmur grading and disease severity can be drawn from the results in this study. Factors such as body condition score, murmur characteristics, and the direction of the MR jet are still considered potential factors that could impact murmur grading, but further research with a larger study population is needed.

Keywords: murmur grading, grading of heart murmurs, factors impacting murmur grading, utility of murmur grading scales, myxomatous mitral valve disease, MMVD, dog

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Abbreviations

ACVIM	American College of Veterinary Internal Medicine
CHF	Congestive heart failure
CKCS	Cavalier King Charles Spaniel
FS	Fractional shortening
LA/Ao	Left atrial-to-aortic ratio
LVIDDN	Normalized left ventricular internal diameter in diastole
LVIDSN	Normalized left ventricular internal diameter in systole
MMVD	Myxomatous mitral valve disease
MR	Mitral regurgitation
PMI	Point of maximum intensity
TR	Tricuspid regurgitation

1. Introduction

Myxomatous mitral valve disease (MMVD) is the most common heart disease in dogs (Detweiler & Patterson 1965b; Whitney 1974). The disease causes degenerative lesions in the mitral valve (other valves can also be affected), resulting in mitral valve insufficiency (or mitral regurgitation, MR) (Ljungvall & Häggström 2017; Ware & Ward 2020a). As the disease slowly progresses, the MR worsens and eventually causes cardiac enlargement in many dogs (Detweiler & Patterson 1965b; Whitney 1974; Borgarelli *et al*. 2008; Ware & Ward 2020a). The first sign of MMVD is usually a heart murmur (caused by the MR) (Keene *et al*. 2019). In most cases, the murmur is discovered long before clinical signs, such as signs related to congestive heart failure (CHF), potentially develop. The preclinical period can be prolonged with medical treatment (Boswood *et al*. 2016; Keene *et al*. 2019). Murmur grading and, primarily, diagnostic imaging techniques can be used to determine whether the patient would benefit from medical treatment or not (Keene *et al*. 2019).

Many veterinarians use a 6-level scale to grade heart murmurs (Prošek 2017; Rishniw 2018; Ware & Ward 2020b). The grades of the 6-level scale are, however, defined differently in various literature (Kvart & Häggström 2002; Smith *et al*. 2006; Prošek 2017; Ware & Ward 2020b). Studies have shown a relatively good agreement between murmur grade and severity of disease on echocardiography for MMVD (Häggström *et al*. 1995; Pedersen *et al*. 1999; Ljungvall *et al*. 2009, 2014; Franchini *et al.* 2021). However, the agreement is not complete. For instance, a dog with a moderate intensity murmur might have mild MMVD lesions on the echocardiogram (Ljungvall *et al*. 2014).

The aim of this study was to evaluate potential factors, such as dog and MR characteristics, that might impact murmur assessment, as well as to evaluate the utility of various murmur grading scales in a group of dogs with MMVD. Evaluation of the utility of various murmur grading scales could potentially help optimize existing murmur grading scales. Moreover, knowledge about factors impacting murmur grading could potentially lead to a better understanding of the association between murmur grades and disease severity (based on echocardiographic assessment).

2. Literature Review

2.1. Physiology of heart valves

The heart valves have a vital function in preventing blood from flowing backward (Sacks *et al.* 2009; Sjaastad *et al*. 2010). The atrioventricular valves (mitral and tricuspid valves) separate each atrium and ventricle (Sacks *et al.* 2009; Sjaastad *et al*. 2010). The semilunar valves (aortic and pulmonic valves) separate the aorta and pulmonary artery from the left and right ventricle, respectively (Sacks *et al.* 2009; Sjaastad *et al*. 2010). The closing of the atrioventricular and semilunar valves produces the first and second heart sounds (S1 and S2), respectively (Sjaastad *et al*. 2010). Differences in the hydrostatic pressure on each side of the various valves determine their opening and closing (Sjaastad *et al*. 2010). The atrioventricular valves open when the pressure in the atria exceeds the pressure in the ventricles. Contraction of the ventricles results in intraventricular pressure exceeding the pressure in the atria, and the atrioventricular valves will close. The increased intraventricular pressure will also exceed the pressure in the aorta and pulmonary artery, which results in the opening of the semilunar valves. As the intraventricular pressure decreases as the ventricles relax, the semilunar valves close.

2.2. Sound and human hearing

Sound originates from vibrations (Smith *et al*. 2006; Ahlström 2008). Vibrations produced in the heart and great vessels generate heart sounds and murmurs (Smith *et al*. 2006). Sound vibrations consist of waves, which in turn consist of areas of varying pressures (Smith *et al*. 2006; Ahlström 2008).

The intensity and frequency of a sound are important for sound interpretation and will be explained below.

2.2.1. Intensity and loudness

The magnitude of the sound waves determines the intensity of a sound (Smith *et al*. 2006). Moreover, the intensity at a specific location is influenced by several factors (Smith *et al*. 2006; Ahlström 2008): Firstly, the intensity of the sound at its origin (for instance, in the heart). Secondly, the distance the sound must travel to reach the listener (for instance, from the heart to an auscultator's ears) because the intensity is inversely proportional to the square of the distance. Thirdly, the homogeneity and density of the structures the sound must pass (for instance, the various structures in the thorax). Sound waves can be reflected and absorbed by various structures (Smith *et al*. 2006; Ahlström 2008). The perceived intensity is termed ‘‘loudness'', and is not linearly related to the physical intensity (this will be further explained below, see 2.2.3.) (Ahlström 2008).

2.2.2. Frequency and pitch

The number of vibrations per second determines the frequency of a sound (Smith *et al*. 2006). The SI unit is Hertz (Hz). The perceived frequency is named ‘‘pitch'' (Ahlström 2008). Below 1000 Hz, frequency and pitch are basically the same (Ahlström 2008). However, above 1000 Hz, the frequency must increase more in order for the change in perceived pitch to be the same.

2.2.3. Interpretation of sound

How one interprets the intensity of a sound (for instance, a murmur) is dependent on both the sound itself and the human hearing. The perceived intensity (or loudness) of a sound is determined by the physical intensity (or sound pressure, measured in dB), as well as the frequency (Smith *et al*. 2006; Sjaastad *et al*. 2010). The frequency is an essential factor because the sensitivity of the human ear differs depending on the frequency (Smith *et al*. 2006). The hearing threshold for a certain frequency is the weakest sound pressure a person is able to hear at that specific frequency (Sjaastad *et al*. 2010).

In young people with normal hearing capability, the audible frequency range is between 20-20000 Hz (Sjaastad *et al*. 2010; Oxenham 2018). However, this range starts to narrow, approximately, after 20 years of age, and it is the ability to detect high frequencies that decreases (Sjaastad *et al*. 2010). In an adult with normal hearing capability, the audible frequency range is approximately 20-14000 Hz (Smith *et al*. 2006). The optimal frequency range for human hearing is 1000-4000 Hz (according to Sjaastad *et al*. (2010)) or 1000-5000 Hz (according to Smith *et al*. (2006)). In this frequency range the audibility threshold is 0 dB (Sjaastad *et al*. 2010). For reference, most sounds during normal speech range between 200-5000 Hz, and the volume is usually 50-60 dB (Sjaastad *et al*. 2010). Many heart sounds and murmurs are not audible due to their low intensity or low frequency (Ware & Ward 2020b). A majority of the clinically relevant heart sounds and murmurs appear in a frequency range between 20-500 Hz (on occasion up to 1000 Hz) according to Smith *et al.* (2006) or 10-1000 Hz according to Ahlström (2008).

When a sound has a frequency within the optimal range, this sound will have a higher perceived intensity compared to a sound with the same physical intensity that has a lower frequency (Smith *et al*. 2006). The hearing sensitivity gradually decreases below 1000 Hz, making it more difficult to hear and correctly interpret sound intensity (Smith *et al*. 2006). A high-intensity sound with a frequency in the lower range can, therefore, be perceived as soft and difficult to hear.

Furthermore, hearing sensibility can vary between individuals and some aspects of hearing can be improved with training (more specifically, to be able to differentiate two sounds that are separated by a short amount of time and to accurately be able to interpret the shape or quality of a sound) (Smith *et al*. 2006).

2.3. Auscultation

2.3.1. The stethoscope

A correctly designed stethoscope and knowledge about how to use it are essential for optimal auscultation. The principal parts of a stethoscope include the chest piece, the tubing, the headpiece, and the earpiece tips (Smith *et al*. 2006; 3M Littmann Stethoscopes n.d.). There are different kinds of chest pieces. Some consist of a single-sided combined bell and diaphragm, which with varying pressure on the chest piece enables the listener to hear sounds with different frequencies (Smith *et al*. 2006; 3M Littmann Stethoscopes n.d.). There are also two-sided chest pieces; with one side being the bell and the other the diaphragm, and electronic stethoscopes (Smith *et al*. 2006; 3M Littmann Stethoscopes n.d.).

The bell is used to listen to both high (100-1000 Hz) and low (20-100 Hz) frequencies (Smith *et al*. 2006). However, high-frequency parts of a mixed frequency sound (for instance, a murmur) may be concealed (and perceived as absent or dull) by high-intensity, low-frequency parts of the same murmur. The diaphragm, which attenuates low-frequency sounds and selectively transfers high-frequency sounds, is used to hear higher frequencies better (Smith *et al*. 2006). Light pressure is applied when using the bell, and firm pressure is applied when using the diaphragm (Smith *et al*. 2006).

Optimal tubing reduces noise and facilitates ideal transmission of sound (Smith *et al*. 2006). The length is important as shorter tubing attenuates sounds of high frequency to a smaller degree than longer tubing.

The headpiece and earpiece tips should fit comfortably (Smith *et al*. 2006). To match the direction of the ear canals, the earpieces should have a slight forward angulation (Smith *et al*. 2006). It is also important that both the tension in the headpiece and the forward angulation of the earpieces are adjustable to fit different individuals (Smith *et al*. 2006). Moreover, the earpiece tips should fit well to minimize leakage of sound and facilitate comfort (Kvart & Häggström 2002; Smith *et al*. 2006).

2.3.2. Factors influencing auscultation

There are many factors that can make auscultation more challenging, including respiratory sounds, sounds from the gastrointestinal system, muscle twitching, shivering, fur rubbing against the stethoscope, the animal not cooperating, and sounds in/from the exam room (Kvart & Häggström 2002; Ware & Ward 2020b). Auscultation should, therefore, be carried out in a quiet room, and the animal should ideally be standing so the heart falls into its normal position.

In animals with thin chests and animals with enhanced ventricular contraction (for instance, due to excitement), the loudness of all heart sounds can be increased (Smith *et al*. 2006). Examples of conditions that decrease loudness are obesity and decreased ventricular contraction (Smith *et al*. 2006).

According to Prošek (2017) the intensity of a murmur at the chest wall is influenced by the direction of the valve regurgitation jet, the characteristics of the structures between the jet and the auscultation area, as well as murmur frequency.

2.4. Heart murmurs

Saunders Comprehensive Veterinary Dictionary defines a murmur as ‘‘an auscultatory sound which results from vibration of turbulent blood flow’’ (Studdert *et al*. 2012, p. 728).

2.4.1. Origin and types of heart murmurs

Heart murmurs usually occur due to turbulence in blood flow in the heart or great vessels (Kvart & Häggström 2002; Smith *et al*. 2006; Studdert *et al*. 2012; Prošek 2017). This can be a result of blood flowing backward (regurgitation) through a defect valve (which is the case in MMVD), abnormal shunting of blood from either side of the heart to the other (septal defects), blood flow through a narrow valve (stenosis), decreased viscosity of blood (for instance, anemia) or high flow rate through valves (Kvart & Häggström 2002; Tilkian & Conover 2001 see Ahlström 2008). The development of heart murmurs can be described by Reynold's number (Studdert *et al*. 2012). The likelihood of turbulent blood flow (and, therefore, the development of a heart murmur) increases with increased velocity of blood flow, as well as with abrupt changes in vessel diameter (Studdert *et al*. 2012). Additionally, decreased viscosity and decreased density of the blood also increase the likelihood of turbulence. Turbulent blood flow results in a high Reynold's number, while a laminar blood flow results in a low Reynold's number (Ahlström 2008).

Murmurs can be divided into pathologic and non-pathologic murmurs (Marriott 1992 see Côté *et al*. 2015; Ware & Ward 2020b). Pathologic murmurs originate from structural cardiac disease. Non-pathologic murmurs can be further divided into functional or innocent murmurs. Functional murmurs occur due to physiological reasons (for example, anemia), whereas innocent murmurs are soft murmurs that can be heard in animals, without structural cardiac abnormalities or physiological changes.

2.4.2. Description of heart murmurs

Murmurs can be described according to their timing, intensity (see 2.5), point of maximum intensity (PMI), radiation, and their pitch and quality (Ware & Ward 2020b). The timing of murmurs can be described as systolic (S1 to S2), diastolic (S2 to S1), or continuous (begins in systole and continues through all or part of diastole) (Kvart & Häggström 2002; Ware & Ward 2020b). The timing can be further specified - for instance, holosystolic or holodiastolic. The PMI can be described by valve area (mitral, aortic, pulmonic, tricuspid) or intercostal space or by words like the apex or the base of the heart (Ware & Ward 2020b). The PMI can also be described by which side of the thorax (left or right) it is located. Murmur radiation is described by where the murmur can be auscultated beyond its PMI, for instance, multiple valve areas (Ware & Ward 2020b). The sound characteristics of a murmur are described by its pitch and quality, which are determined by murmur frequency and the clinician's subjective assessment (Ware & Ward 2020b). The quality can be harsh, soft, or musical (Kvart & Häggström 2002). The frequency of the murmur can be predominantly low (50-100 Hz), medium (100-200 Hz), or high (400-500 Hz). Murmurs can also consist of mixed-frequency sounds (Prošek 2017).

Phonocardiographic configuration is another way to describe a murmur (Ware & Ward 2020b). When recording the heart sounds and murmur with a phonocardiograph, the heart sounds and murmur are graphically displayed (Kvart & Häggström 2002). Different murmurs have different phonocardiographic shapes, including plateau-shaped (begins at S1 and generally maintains the same intensity throughout systole), diamond-shaped (a murmur that is initially soft and then gradually increases in intensity in mid-systole, followed by gradually decreasing in intensity), systolic/diastolic decrescendo (the initial intensity gradually decreases), and continuous (a murmur that is present during systole and continues into part of or during all of diastole) (Ware & Ward 2020b).

2.5. Grading of heart murmurs

2.5.1. History of murmur grading

There have been different murmur grading systems proposed throughout history. In a review article about the history of murmur grading in humans and animals, the author stated that the first murmur grading scale (for humans) was originally created by A.R. Freeman and Samuel Levine (Rishniw 2018) and consisted of 6 grades describing murmur intensity (Freeman & Levine 1933 see Rishniw 2018). A grade 1 murmur was defined (Freeman & Levine 1933 see Rishniw 2018, p. 226) by its intensity (the faintest murmur) and by its duration (''continue after the first heart sound and well into systole''). Grade 2 was defined as ''a slight murmur''. Freeman and Levine mentioned (within the definition) that the auscultators in their study were experienced and auscultated with great care. Therefore, a grade 2 murmur is probably what a general physician considers ''a faint systolic murmur'' and that grade 1 murmurs probably usually are not heard or are overlooked in the routine general practice. Grades 3 through 6 were defined by the following words: ''moderate intensity'', ''loud'', ''very loud'', and ''loudest possible''. Moreover, Levine (1961) provided more detailed information concerning the different grades in a subsequent article. In this article, Levine (1961, p. 261) stated that a grade 1 murmur is a faint murmur ''heard only after several seconds of auscultation'', while a grade 2 murmur is faint but ''heard immediately''. Furthermore, murmur grade 6 is audible with the stethoscope slightly removed from the skin (not touching the skin). A grade 5 murmur was further defined as a loud murmur that can be heard if the edge of the stethoscope is touching the skin, but not when the stethoscope is lifted off the skin. Moreover, Levine (1961, p. 261) stated that the different grades could be described accordingly: ''very slight, slight, moderate, loud, very loud, and loudest possible murmurs''.

The first grading scale for dogs consisted of 5 levels (Rishniw 2018). The 5-level scale was originally published by David Detweiler in 1959 (Detweiler 1959 see Rishniw 2018) and then reproduced in an article by Allen Hahn (Hahn 1962 see Rishniw 2018). The same scale was later published by David Detweiler and Don Patterson (Detweiler & Patterson 1965a, 1967). Rishniw (2018) stated that Levine or others were not, however, credited in any of the publications of this 5-level scale, although it was similar to the scales used for people at this time. In this 5-level scale (Detweiler & Patterson 1965a, p. 324, 1967, p. 196), a grade 1 murmur was defined as the ‘‘softest audible murmur’’. A grade 2 murmur was described as ‘‘a faint murmur’’, that could be distinctly heard after a few seconds. A grade 3 murmur was a murmur that could be heard immediately and over a ‘‘fairly large area’’. A grade 4 murmur was defined as the ‘‘loudest murmur’’, not audible when the chest piece was slightly removed from the chest wall, while a grade 5 murmur remained audible.

In the textbook *Canine Cardiology*, Ettinger and Suter presented the 5-level scale by Detweiler and Patterson but also suggested a 6-level scale, almost exactly defined like the Levine-scale (Ettinger & Suter 1970 see Rishniw 2018). However, Ettinger and Suter did not include precordial thrills in the definitions of murmur grades V-VI/VI, but they stated that a thrill always accompanies a great grade V or VI murmur. Furthermore, the intensities were divided into soft, medium, and loud. Rishniw (2018) stated that most veterinarians have used this murmur grading scale for the last 40 years.

2.5.2. The 6-level murmur scale today

The definitions of four different textbooks’ murmur grading scales are presented below. In summary, none of them have the exact same definitions.

In the book *Rapid Interpretation of Heart and Lung Sounds – A Guide to Cardiac and Respiratory Auscultation in Dogs and Cats*, the authors described a 6-level scale for grading the intensity of heart murmurs in dogs and cats (Smith *et al*. 2006). The authors mentioned that the grading system is not universally accepted. They defined the various grades as follows (Smith *et al*. 2006, p. 43):

> ‘‘I/VI signifies a very soft murmur heard only in a quiet room after a period of concentrated listening over the point on the chest where the murmur is heard.
>
> II/VI is a soft murmur that is audible as soon as the stethoscope chestpiece is appropriately placed at the PMI. A II/VI murmur does not radiate widely from the point on the chest where it is heard best.
>
> III/VI is louder, heard easily some distance away from its PMI (but not generally audible on the opposite side of the chest).
>
> IV/VI signifies a loud murmur, radiating widely (often including the opposite side of the chest), but not associated with a palpable precordial thrill.

> V/VI designates a very loud murmur associated with a palpable precordial thrill that always marks its PMI on the chest wall.
>
> VI/VI designates an extremely loud murmur that not only is associated with a palpable precordial thrill, but also can be heard without stethoscope or with the stethoscope removed from the chest wall.''

Kvart and Häggström (2002) defined the 6-level heart murmur scale slightly differently. The murmurs were defined as being of low intensity (grades 1-2), moderate intensity (grade 3), and high intensity (grades 4-6). Unlike Smith *et al.* (2006), Kvart and Häggström did not include the criterion that the murmur can be heard without a stethoscope (grade 6) in their definition. Furthermore, the grade 3 murmur was only defined by its intensity. Otherwise, the definitions are similar. Kvart and Häggström (2002, p. 16) defined the various murmur grades accordingly:

> ''Grade 1: A low intensity murmur heard in a quiet environment only after careful auscultation over a localized cardiac area.
>
> Grade 2: A low intensity murmur heard immediately when the stethoscope is placed over the point of maximal intensity.
>
> Grade 3: A murmur of moderate intensity.
>
> Grade 4: A high intensity murmur that can be auscultated over several areas without any palpable precordial thrill.
>
> Grade 5: A high intensity murmur with a precordial thrill.
>
> Grade 6: A high intensity murmur with a palpable thrill that may even be heard when the stethoscope is slightly lifted off the chest wall.''

In the book *Small Animal Internal Medicine*, the authors described grade 1 and grade 2 murmurs practically the same as Smith *et al.* (2006) (Ware & Ward 2020b). The definitions for grades 3-6 were similar. However, a grade 3 murmur was defined as a moderate-intensity murmur, grades 4-5 were defined as ''loud'' and grade 6 was described as ''very loud''. Furthermore, Ware and Ward included that grade 5 and 6 murmurs usually radiate widely. Like Kvart and Häggström (2002), Ware and Ward did not include the criterion that the murmur can be heard without a stethoscope (grade 6) in their definition. Ware and Ward (2020b, p. 10) defined the 6-level murmur scale as follows:

> Grade 1: ''Very soft murmur; heard only over its site of origin, after prolonged listening in quiet surroundings''
>
> Grade 2: ''Soft murmur but easily heard over its site of origin (usually a particular valve area)''

Grade 3: ‘‘Moderate-intensity murmur; usually radiates to other precordial/valve areas too’’

Grade 4: ‘‘Loud murmur but without a precordial thrill; radiates widely and usually can be heard over most precordial regions’’

Grade 5: ‘‘Loud murmur with a palpable precordial thrill; radiates widely and usually can be heard clearly over all precordial regions’’

Grade 6: ‘‘Very loud murmur with a palpable precordial thrill; radiates widely, generally is heard clearly over all precordial areas, and also can be heard with the stethoscope chestpiece lifted slightly (~ 1 cm) from the chest wall (at the murmur PMI)’’

Finally, in the *Textbook of Veterinary Internal Medicine*, Prošek (2017) presented a murmur grading system consisting of 6 levels similar to those of the other textbooks. Like other authors, Prošek did not include the criterion that the murmur can be heard without a stethoscope (grade 6) in their definition. Similar to Kvart and Häggström (2002), the grade 3 murmur was only defined by its intensity. Prošek (2017, p. 222) defined the 6-level scale as follows:

‘’Grade 1: Very soft, localized murmur detected in a quiet room after intently listening for a few minutes

Grade 2: Soft murmur but easily heard after a few seconds

Grade 3: Moderate-intensity murmur

Grade 4: Loud murmur but not accompanied by a palpable thrill (vibration)

Grade 5: Loud murmur accompanied by a palpable thrill

Grade 6: Very loud murmur that produces a palpable thrill still audible after stethoscope is removed from the chest’’

2.5.3. Challenging the 6-level murmur scale

Several articles have challenged the current grading system. In one study, the authors investigated if using the heart sounds as an internal reference would improve the grading of heart murmur intensity in humans (Keren *et al*. 2005). The internal reference grading system consisted of 3 levels (Keren *et al*. 2005, p. 330): ‘‘Clearly softer than the heart sounds’’, ‘‘Approximately equal in intensity to the heart sounds’’, and ‘‘Clearly louder than the heart sounds’’. The study included a total of 100 medical students, residents, and pediatric attending physicians. The participants described their current way of grading heart murmurs – a majority used a system very similar to the Levine system. However, none of the participants defined grades 1-3 exactly like Levine. The participants were asked to grade several murmurs with their current grading scale (murmur intensities ranged between 0-3/6). One group was then taught the 3-level murmur grading scale (the control group continued with their murmur grading scale). Statistical analyses were performed for the various experience levels and different murmur grades (0-3). Results showed that grading accuracy improved when using the internal reference system, in attending physicians and students, as well as for grade 2 murmurs. Grading consistency improved in attending physicians, as well as for murmur grades 2 and 3. However, no statistically significant improvement in interobserver agreement was seen.

In another study, Ljungvall *et al*. (2014) examined records of 578 small dogs with MMVD and compared murmur intensity with findings on echocardiography and clinical disease. Results showed that when using a 4-level murmur classification system, consisting of the classifications/grades soft (grades 1 and 2), moderate (grade 3), loud (grade 4), and thrilling (grades 5 and 6), no important information about probability of CHF, cardiac remodeling or pulmonary hypertension was lost. In another study, the authors compared a 6-level grading system to the 4-level grading system used by Ljungvall *et al.* (however, the word ‘‘palpable’’ was used instead of ‘‘thrilling’’) (Caivano *et al*. 2018). The results indicated that the 4-level grading system for dogs with pulmonic or subaortic stenosis, could differentiate stenosis severity and no clinical information was lost compared to when using a 6-level grading system.

Moreover, there are several studies that have used variations of the 6-level murmur scale in their statistical analyses. Three studies separated murmurs into low intensity (grades 1 and 2), moderate intensity (grades 3 and 4) and high intensity (grades 5 and 6) (Häggström *et al*. 1995; Kvart *et al*. 2002; Ljungvall *et al*. 2009). Furthermore, in a study by Borgarelli *et al.* (2008), murmur intensity was divided into two groups (low intensity and moderate to high intensity).

2.5.4. Factors that might impact murmur grading

No studies investigating if age, body weight, BCS, sex, or chest shape impact murmur grading were found while reviewing the literature.

Breed

In a retrospective study, including 1088 dogs of 106 various breeds (excluding mixed breed dogs), the authors concluded that breed does not affect the association between murmur intensity and severity of subaortic and pulmonic stenosis (Rishniw *et al*. 2019). A difference was seen between Boxers and French Bulldogs concerning moderate murmurs (French Bulldogs had higher pressure gradients on echocardiography, indicating more severe stenosis). However, the same difference was not observed between these breeds concerning loud or palpable murmurs (the 4-level scale described above was used). The authors discussed that there were only 11 French Bulldogs, why a conclusion about if this was a true difference could not be made. Approximately 30% of the dogs included in the study by Rishniw *et al.* were included in the previously mentioned study by Caivano *et al.* (2018). Caivano *et al.* mentioned in the discussion that no effect of breed was observed on the auscultatory characteristics of murmurs in this study.

Heart rate and acute stress

When the sympathetic nervous system is activated, for instance, when an animal is stressed, the heart rate will increase, as well as the force of contraction of the heart, and the blood pressure will be elevated due to vasoconstriction (Sjaastad *et al*. 2010).

In a study evaluating interobserver variation and effects of stress testing on low-intensity murmurs in boxer dogs, murmur gradings were higher after exercise, compared with rest, for 5 of the 6 auscultators (Höglund *et al*. 2004). In many of the dogs with a low-intensity murmur at rest, the intensity increased at least one grade after exercise. Furthermore, a murmur was auscultated after exercise in over half of the dogs that had no murmur initially. More dogs that initially had either no murmur or a grade 1 murmur increased in murmur intensity after exercise, than dogs with grade 2 or 3 murmurs at rest. The two most experienced auscultators graded murmurs higher after exercise in more dogs than less experienced auscultators.

Similar results were seen in another study evaluating observer variation (6 different auscultators) and effects of physical maneuvers on auscultation in Cavalier King Charles Spaniels (CKCS) with mild MR (Pedersen *et al*. 1999). The murmur grade was higher in 33% and 38% of the total auscultations after two different stress tests, respectively. More dogs with initial low-intensity murmurs received a higher murmur grade after the two different stress tests (respectively), than dogs with grade 3 or 4 murmurs. Grading a higher intensity murmur after the two different stress tests (respectively), correlated with higher experience level. In 20% and 31% of dogs with no initial murmur, a murmur was auscultated after the two stress tests, respectively. Furthermore, more dogs with a higher intensity level (according to the two most experienced observers) after one of the two different stress tests, belonged to the group of dogs with an increased heart rate, compared to dogs with decreased or unaltered heart rate after this particular stress test.

Drugs

Various drugs can affect the cardiovascular system. In the textbook *Rang & Dale's Pharmacology*, the effects of various drugs on heart function are divided into three main aspects of heart function (Rang *et al*. 2016): 1) rate and rhythm, 2) metabolism and blood flow, and 3) myocardial contraction.

2.6. Myxomatous mitral valve disease

Myxomatous valve disease is the most common cardiac disorder in dogs (Detweiler & Patterson 1965b; Whitney 1974). The disease is most common in small to mid-sized dogs, with the highest prevalence in the CKCS (Thrusfield *et al*. 1985; Häggström *et al*. 1992; Beardow & Buchanan 1993; Egenvall *et al*. 2006). In CKCS and dachshunds, the disease seems to have a polygenic inheritance (Swenson *et al*. 1996; Olsen *et al*. 1999; Zachary 2017). The prevalence of disease increases with age (Detweiler & Patterson 1965b; Jones & Zook 1965; Detweiler *et al*. 1968; Whitney 1974; Häggström *et al*. 1992).

The lesions are usually the most severe in the mitral valve (Detweiler & Patterson 1965b; Luginbühl & Detweiler 1965 see Whitney 1967; Detweiler et al. 1968; Whitney 1974). The disease is, therefore, often referred to as myxomatous mitral valve disease (MMVD) (Ware & Ward 2020a). The mitral valve consists of two leaflets: the anterior and the posterior leaflet (Kogure 1980; Ware & Ward 2020a). Furthermore, the tricuspid valve is also affected in many dogs (Detweiler & Patterson 1965b; Luginbühl & Detweiler 1965 see Whitney 1967; Detweiler *et al*. 1968; Whitney 1974). The aortic and pulmonic valves may also be affected, but it is not as common and are seldomly of clinical importance.

The degenerative lesions prevent the edges of affected valves from coapting properly when they close (Ljungvall & Häggström 2017; Ware & Ward 2020a). This causes regurgitation, and blood will leak through the defect valve from the ventricle into the atrium. The disease usually progresses slowly, and the severity increases with age (Detweiler & Patterson 1965b; Whitney 1974; Borgarelli *et al*. 2008). The preclinical period is, therefore, usually comparably long. As the disease progresses, the regurgitation worsens and eventually causes volume overload of the adjacent atrium and ventricle, resulting in enlargement of the atrium and ventricle (Detweiler & Patterson 1965b; Ware & Ward 2020a).

Congestive heart failure and other complications, such as rupture of chordae tendineae, atrial rupture, arrhythmias, and pulmonary hypertension, may occur in MMVD dogs (Ljungvall & Häggström 2017; Ware & Ward 2020a). However, many affected dogs will never develop any complications due to their disease. Signs of early CHF might include tachypnea, coughing with exertion, and reduced exercise tolerance (Ware & Ward 2020a). Clients are often instructed to monitor sleeping/resting respiratory rate of their dog at home, as an increase in this type of respiratory rate is an early indication of the onset of pulmonary edema (Ware & Ward 2020a). Furthermore, severe pulmonary edema, which can develop acutely or progressively, results in respiratory distress, often accompanied by a cough (Ware & Ward 2020a). In dogs with advanced MMVD, syncope and weakness might be seen (Ware & Ward 2020a). Moreover, right-sided CHF (which most commonly is seen in dogs with severe tricuspid regurgitation and/or pulmonary hypertension) can cause respiratory distress due to pleural effusion, abdominal distension caused by ascites and/or hepatomegaly, gastrointestinal clinical signs, and edema in peripheral tissues (Ware & Ward 2020a).

In dogs with MMVD, a left apical holosystolic murmur is common (Häggström *et al*. 1995; Pedersen *et al*. 1999; Kvart & Häggström 2002). However, dogs with minimal to mild regurgitation can have no murmur or have a murmur only in early systole. As disease progresses, the murmur usually increases in intensity and duration.

Echocardiography is a useful method for diagnosing and monitoring MMVD (Ljungvall & Häggström 2017). Features that can be evaluated by echocardiography in a dog with suspected or previously confirmed MMVD include valve morphology, severity of MR, ventricular function, atrial enlargement, and ventricular enlargement (Ware & Ward 2020a).

2.6.1. Classification according to ACVIM

In 2009 the American College of Veterinary Internal Medicine (ACVIM) Specialty of Cardiology consensus panel adapted a staging system for heart disease and CHF in dogs with MMVD (Atkins *et al*. 2009). The article also included guidelines for the diagnosis and treatment of dogs with MMVD. An updated consensus statement was published in 2019 (Keene *et al*. 2019).

The four stages (A-D) included in the staging system are described below (Keene *et al*. 2019):

Stage A – includes dogs with high risk of developing MMVD, with no current detectable structural heart disease.

Stage B – includes dogs with structural heart disease whose history does not include clinical signs due to CHF. Stage B is further divided into two substages: B1 and B2. *Stage B1* includes dogs with no echocardiographic or radiographic signs of left atrial or ventricular enlargement, or with enlargement that is not severe enough to qualify for Stage B2. *Stage B2* includes dogs with radiographic or echocardiographic signs of left atrial and ventricular enlargement that meet inclusion criteria used in the EPIC study (Boswood *et al*. 2016). Medical treatment is indicated in stage B2 dogs.

Stage C – includes dogs with current or previous clinical signs of CHF.

Stage D – includes dogs with clinical signs of CHF, which are refractory to standard treatment. To manage the disease, advanced or specialized treatment is necessary.

2.6.2. Diagnosis and staging of disease severity

According to the ACVIM guidelines (Keene *et al*. 2019) for diagnosis and categoryzation of dogs into stage B1 and stage B2, the panel states that MMVD usually is detected by auscultation of a heart murmur, for instance, during a routine veterinary exam. The panel recommends thoracic radiography, to measure blood pressure and to perform echocardiography. Dogs in stage B1 will either have no echocardiographic or radiographic signs of left atrial or ventricular enlargement and have normal left ventricular systolic function, or the enlargement is not severe enough to qualify for stage B2 (see below).

To classify a dog as stage B2, the dog should have a murmur intensity ≥3 (Keene *et al*. 2019). Furthermore, the echocardiographic measurement left atrial-to-aortic ratio (LA/Ao) in the right-sided short axis view in early diastole should be ≥1.6, and the echocardiographic measurement normalized left ventricular internal diameter in diastole (LVIDDN) should measure ≥1.7. Moreover, the breed-adjusted radiographic measurement vertebral heart size (VHS) should be >10.5. Echocardiography is considered the most reliable tool to identify dogs in stage B2 (Keene *et al*. 2019). These measurements are the same as the inclusion criteria used in the EPIC study, in which dogs that received pimobendan had a prolonged preclinical period, compared to dogs that received placebo (Boswood *et al*. 2016). Therefore, the ACVIM panel recommends treating dogs in stage B2 with pimobendan (Keene *et al*. 2019).

Dogs in stage C should show or have shown clinical signs of CHF caused by MMVD (Keene *et al*. 2019). Patient signalment, history, and physical examination might be useful in evaluating the cause of clinical signs. Furthermore, echocardiography (ideally) and radiography can be performed. The panel also recommends obtaining blood- and urine samples for testing, as well as measuring blood pressure. Serum NT-proBNP might also be useful. Treatment for dogs in Stage C depends on disease severity and whether the dog is in acute CHF or not, but pimobendan and furosemide are always recommended for these dogs.

Classifying dogs as stage D includes the same steps as classifying dogs as stage C, with the addition that they are refractory to standard treatment (standard treatment being the treatment recommended for patients in stage C) (Keene *et al*. 2019).

2.7. Murmur grade and MMVD severity

Several studies have shown a positive association between murmur grade and severity of MMVD. In the previously mentioned study by Ljungvall *et al*. (2014), results showed that murmur intensity reflects disease severity in small dogs with MMVD. The above-mentioned 4-level classification system, including classifications/grades soft (grade 1-2), moderate (grade 3), loud (grade 4), and thrilling (grades 5-6), was used. Most dogs (90%) with soft murmurs had mild disease with no cardiac remodeling, which only was the case for 8% of dogs with thrilling murmurs. In the group of dogs with soft murmurs, none had CHF. The probability of having cardiac remodeling, CHF, or pulmonary hypertension increased with increasing murmur intensity; the highest probability, therefore, being in dogs with thrilling murmurs. The authors conclude that soft murmurs indicate mild disease, while thrilling murmurs indicate more advanced disease. Regarding moderate (grade 3) and loud (grade 4) murmurs, the authors conclude that no accurate conclusions about disease severity can be made from only auscultation.

In another study, including 1245 dogs $\geq$2 kg and $\leq$25 kg in stages B1 and B2, using the same murmur classification system as Ljungvall *et al*. (2014), results showed that dogs with higher intensity murmurs were more likely to be in stage B2, compared to stage B1 (Wilshaw *et al*. 2021). The dogs' probability of being in stage B2 increased with increasing murmur grade, but there was no significant difference in likelihood of being in stage B2 when loud and thrilling murmurs were compared.

Similar results have been found in studies using 6-level murmur scales. In the previously mentioned study by Pedersen *et al*. (1999), mean murmur intensity was associated with jet size on echocardiography. When evaluated for different observers, the more experienced observers' murmur gradings correlated with the size of the jet, while the two least experienced observers' gradings did not. 81% (29/36) of dogs with murmur grades 3 or 4, had a moderate or large jet. The study included 57 CKCS.

In another study, murmur intensity (1-6) was one of the significant predictors of an increase in left atrial diameter (Olsen *et al*. 2003). The increase in left atrial diameter was greater in dogs with grade 2 and grade 3 murmurs, compared to dogs with grade 1 murmurs or no murmur. No difference was seen between dogs with grade 1 murmurs and dogs with no murmurs. The study included 131 dachshunds, which were reexamined three years after an initial examination (190 dachshunds were examined at the initial examination).

Three studies separated murmurs into low intensity (grades 1 and 2), moderate intensity (grades 3 and 4) and high intensity (grades 5 and 6). In one of these studies, association between murmur intensity and severity of MR was seen (Ljungvall *et al*. 2009). The severity of MR was based on LA/Ao and the jet size. The study included 77 dogs of different breeds, the most common breed being CKCS (59/77 dogs). In one of the other studies, including 229 CKCS with MMVD, results showed that dogs with initial moderate or high-intensity murmurs developed CHF quicker (dogs with high-intensity murmurs the quickest) than dogs with an initial low-intensity murmur (Kvart *et al*. 2002). Finally, in the third study, including a total of 79 CKCS (59 with a systolic murmur), increasing heart murmur intensity correlated with increasing disease severity and increasing cardiac size (based on left ventricular end diastolic diameter [LVEDD] and left atrial-to-aortic ratio [LA/Ao]) (Häggström *et al*. 1995).

In a study evaluating various data from history and physical examination in 244 dogs with MMVD and their relation to outcome, results showed that heart murmur intensity was related to outcome (Lopez-Alvarez *et al*. 2015). Specifically, having a heart murmur grade >3/6 was associated with an increased risk of cardiac mortality, compared to having a murmur grade $\leq$3/6. The study included several different breeds with a median body weight of 10 kg (interquartile range 7.6-12.9 kg).

In a retrospective study evaluating various variables and their effect on survival time and prognosis of MMVD, survival time was not significantly affected by murmur intensity (Borgarelli *et al*. 2008). This study included a great variety of breeds of different sizes and with various chest shapes. Murmur intensity was divided into two groups (low intensity and moderate to high intensity). Furthermore, dogs with atrial fibrillation were included in the study. The authors discuss that these above-mentioned factors (many breeds, murmur intensity only divided into two groups, and inclusion of dogs with atrial fibrillation) might have impacted the result. However, in a recent study, including a wide variety of breeds, an association between murmur intensity and disease severity was demonstrated (Franchini *et al*. 2021). In this prospective study, including 6102 dogs with MMVD, murmur intensity had a positive agreement with ACVIM stage and left atrial (LA) enlargement (based on LA/Ao). The 4-level scale mentioned above was used for murmur grading. Dogs in stage B1 more frequently had soft or moderate murmurs than dogs in other stages. Furthermore, dogs lacking a murmur were most likely to be in stage B1. Dogs in stages B2 and C more frequently had loud or thrilling murmurs. Most dogs whose LA was enlarged, had a loud or a thrilling murmur.

Soft murmurs were only found in 3% and 2% of dogs that had mild-moderate and severe enlargement, respectively. Among dogs with normal LA dimensions, 37%, 26% and 5% of the dogs had moderate, loud, and thrilling murmurs, respectively. There was a positive association between ACVIM stage and heart rate (during physical examination).

Severity of MR was associated with murmur intensity (1-6) in a study including 70 mixed breed and purebred dogs (Soares *et al*. 2005). However, no statistical analysis seems to have been performed. The majority of dogs with grade 1 (3 dogs) and grade 2 (3 dogs) murmurs had mild MR (one dog with a grade 2 murmur had moderate MR). Most of the dogs with grade 3 murmurs (17 dogs in total) had mild MR (58.8%), 35.3% had moderate MR, and 5.9% had severe MR. A majority of dogs with grade 4 murmurs (28 dogs in total) had moderate MR (46%), while 32% had mild MR and 22% severe MR. Most dogs with grade 5 (15 dogs in total) and grade 6 (4 dogs in total) murmurs had severe MR (73.3% and 75%, respectively), and the rest had moderate MR.

3. Material and Methods

This prospective study was conducted at the Cardiology Clinic at the University Animal Hospital at the Swedish University of Agricultural Sciences (SLU) in Uppsala, Sweden. The study started in June 2021 and finished in November 2021. Client-owned dogs presenting with a systolic heart murmur at the Cardiology Clinic were recruited to the study if they, based on echocardiographic findings, had a diagnosis of MMVD. Dogs with other cardiovascular diagnoses, systemic disease, or arrhythmias (other than respiratory sinus arrhythmia) were excluded. Only medically indicated examinations were performed, and no examinations were made for the sole purpose of the study.

Information about dog characteristics, data from clinical examination, and echocardiographic findings (specified below) were collected during the visit. The data were collected by both the degree project student (referred to as ‘‘the student’’ below) and the supervisors (further specified below).

No personal information about the owner was collected. However, the medical record number could technically be connected to the owner, but only if searched in the medical record system, which is only accessible to personnel and students at the hospital. The medical record number was not included in the written project. Collected data were stored either at the Cardiology Clinic (which is only accessible to personnel at the hospital with special privileges), not visible or accessible to other clients, or in the student’s home (data not visible or accessible to visitors). Only the student and the supervisors processed the data. The data were not presented in a way that could be linked to an individual dog or dog owner.

The data were subsequently entered into a Microsoft Excel document.

3.1. Dog characteristics

The following information about the dogs was collected during each visit: medical record number (not presented in the written project), date of birth, body weight, body condition score (1-9), date of examination (not presented in the written project), breed, sex (female, male, neutered female, neutered male), chest shape (deep, normal, round), heart rate during auscultation (counted once during auscultation) and any potential medical treatment protocol. These data were collected either by the student or the supervisors. The student confirmed with a supervisor if uncertain about any assessment.

The ACVIM-grade (A, B1, B2 C, D) was determined taking history, all examinations, and echocardiographic variables into account. For dogs in stage C, it was also noted whether the dog was in active (decompensated) CHF or if the dog was in stabilized (compensated) CHF (no current clinical signs).

3.2. Heart murmur

Information about the heart murmur was obtained through auscultation with a stethoscope, as well as from listening with the ear close to the chest wall and by palpation of the precordial area. The auscultation and listening were performed in a quiet room. The student used a 3M Littmann Cardiology IV stethoscope, and the supervisors used an electronic stethoscope (Meditron) and a 3M Littmann Classic III stethoscope.

A list of the data collected is presented below. Points 1-6 were always approved by one of the supervisors. Point 7 was approved by a supervisor if the supervisor had assessed the audibility of the murmur when listening close to the chest wall, without the use of a stethoscope. If the supervisor had not listened close to the chest wall, this assessment was made solely by the student.

1. Assessment of murmur grade: 1-6
2. Assessment of murmur grade: mild, moderate, severe without/with a precordial thrill
3. Assessment of the murmur intensity: very soft, soft, moderate, loud, very loud, extremely loud
4. Precordial thrill at PMI: yes or no
5. Assessment of the PMI and how the murmur radiates (mitral valve area, pulmonic valve area, aortic valve area, tricuspid valve area)
6. Assessment of the audibility of the murmur when the stethoscope was lifted slightly off the chest wall (no contact with the chest wall): yes or no
7. Assessment of the audibility of the murmur when listening close to the chest wall, without the use of a stethoscope, over the PMI: yes or no

3.3. Echocardiography

Echocardiography was performed by one of the three supervisors using a Philips EPIQ 7G ultrasound machine. The following information was collected: heart rate during echocardiography (collected from the simultaneously recorded electrocardiogram), normalized left ventricular internal diameter in diastole (LVIDDN), left atrial-to-aortic ratio (LA/Ao), normalized left ventricular internal diameter in systole (LVIDSN), and fractional shortening (FS). For all affected valves, the grade of regurgitation (mild, moderate, severe) and the velocity of the regurgitation were noted. Additionally, information about the direction of the jet (central, lateral, septal/medial and the angle of the main direction of the jet) was collected for the MR (see *Figure 1*). The angle was measured on the echocardiograms by one of the assistant supervisors (JH) and will be further described below.

3.3.1. Echocardiographic assessments and measurements

The LA/Ao was measured in the right parasternal short axis 2D view in early diastole (Hansson *et al*. 2002). The left ventricular internal diameter in diastole (LVIDd) and left ventricular internal diameter in systole (LVIDs) were measured on the M-mode echocardiogram, which was obtained from the right parasternal short-axis view (Thomas *et al*. 1993). From these measurements, the echocardiographic system automatically calculated LVIDDN and LVIDSN using the following equations (Cornell *et al*. 2004; Boswood *et al*. 2016):

$$LVIDDN = LVIDd(cm)/(body\ weight(kg))^{0,294}$$
$$LVIDSN = LVIDs(cm)/(body\ weight(kg))^{0,315}$$

The FS (%) was derived from these measurements as well, using the following equation ((LVIDd-LVIDs)/LVIDd x 100) (Ware & Ward 2020c).

Furthermore, the mitral valve was subjectively evaluated in the right parasternal long-axis views, as well as the left apical four-chamber view (Thomas *et al*. 1993). These views were also used to subjectively determine the grade of MR with color mode Doppler by assessing the area of the regurgitant jet in relation to the area of the left atrium (Ljungvall *et al.* 2009). The degree of MR was divided into categories as described by Olsen *et al.* (2003), but with the modifications used by Ljungvall *et al.* (2009): no regurgitation, mild (< 30%), moderate (30-50%), and severe (> 50%). All valves were evaluated for regurgitation with color Doppler (Ljungvall *et al*. 2009). Moreover, the peak velocity of the regurgitation was measured with continuous wave Doppler (Ware & Ward 2020c)

The MMVD diagnosis was based on echocardiographic findings, including thickened and/or prolapsed mitral valve leaflets, as well as MR on the color Doppler echocardiogram (Pedersen *et al*. 1996; Olsen *et al*. 2003; Ljungvall *et al*. 2013)

3.3.2. Mitral regurgitation jet direction

The angle of the MR jet was calculated in the left apical four-chamber view. The mitral valve plane was marked out by drawing a line between the hinge points of the mitral annulus, followed by marking out the main direction of the jet starting from its starting point through the defect valve. The echocardiographic system then automatically calculated the angle between these two lines. See *Figure 1*.

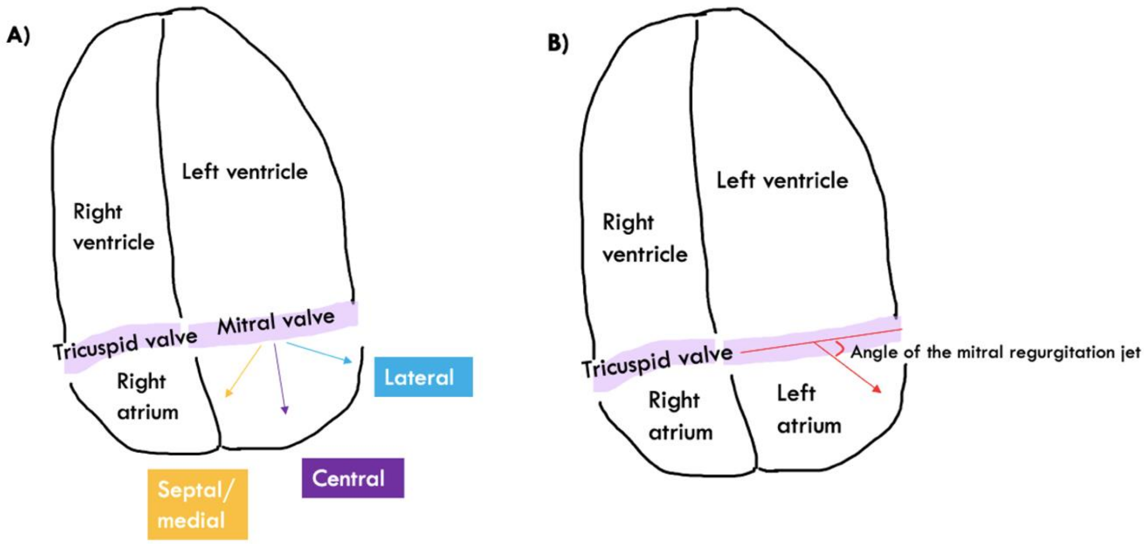

***Figure 1**.*
Illustration of the heart in the left apical four-chamber view.
***A)** Examples of a mitral regurgitation (MR) jet with a lateral (blue arrow), central (purple arrow), and septal/medial (yellow arrow) direction.*
***B)** Calculation of the angle of the MR jet. The mitral valve plane was marked out by drawing a line between the hinge points of the mitral annulus (red line), followed by marking out the main direction of the jet starting from its starting point through the defect valve (red arrow). The echocardiographic system then automatically calculated the angle between these two lines.*

3.4. Statistical analysis

The program JMP Pro 14.00 was used for statistical analysis. Categorical data are mainly presented descriptively as no reliable statistical analysis using Chi-square tests could be performed because the basic assumptions of this test were not fulfilled (one or several cell counts had an expected count <1, or >20% of the cells had an expected count <5). Comparisons between categorical data were performed using the Chi-square test. Subanalyses were performed with pairwise comparisons in cases where the overall p-value was <0.05 and one or both variables included >2 groups using the Fischer’s exact test with Bonferroni correction for multiple comparisons.

Wilcoxon/Kruskal-Wallis tests (rank sums) were performed for comparisons between groups for numerical variables, followed by a one-way ChiSquare approximation. If the probability>ChiSquare value for the ChiSquare approximation was <0.05, a nonparametric comparison for each pair using the Wilcoxon method was performed. For the latter analysis, a Bonferroni adjusted p-value of 0.017 was used for statistical significance.

4. Results

4.1. Dog characteristics

A total of 53 dogs were included in the study. The median body weight was 8.1 kg (interquartile range (IQR) 5.3-10.4), and the median age at examination was 10.7 years (IQR 8.6-12.7). A total of 16 breeds were represented in the study: CKCS (n=19), Mixed breed (n=8), Havanese (n=5), Chihuahua (n=4), Jack Russel (n=3), Dachshund (n=2), Lagotto Romagnolo (n=2), Miniature Schnauzer (n=2), American Cocker Spaniel (n=1), Bolognese (n=1), Chinese Crested Dog (n=1), Drever (n=1), German Spitz/Mittel (n=1), Japanese Spitz (n=1), Pomeranian (n=1) and Shih Tzu (n=1). The breeds were divided into CKCS (n=19) and other breeds (n=34) for statistical analysis. The study group consisted of 37 male dogs (of which 12 were neutered) and 16 female dogs (of which 8 were neutered). Furthermore, 16 dogs (30%) had a normal chest shape, 14 dogs (26%) were deep-chested, and 23 dogs (43%) had a round chest shape. Dogs with BCS 3-7 were represented in the study (n=52). The dogs were divided into normal (BCS 4-5, n=33) and overweight (BCS 6-7, n=18). Only one dog had BCS 3, why this dog was excluded from statistical analyses concerning BCS. The median heart rate during auscultation (n=53) was 120 beats per minute (BPM) (IQR 111-140).

The following drugs were represented in the treatment protocol of the 53 dogs: pimobendan (32%, n=17), furosemide (17%, n=9), meloxicam (n=3), carprofen (n=2), sildenafil (n=1), spironolactone (n=1), paracetamol (n=1), robenacoxib (n=1), benazepril+spironolactone (n=1), bedinvetmab (n=1), ciclosporin (n=1), ophthalmic ciclosporin (n=1) and omeprazole (n=1).

ACVIM stages B1, B2, C and D were represented among the 53 dogs: B1 (n = 19, 36%), B2 (n = 24, 45%), C (n = 7, 13%) and D (n = 3, 5,7%). Because only 3 dogs were in stage D, these dogs were combined with dogs in stage C for statistical analyses.

All data were not possible to measure/collect for all the included dogs, explaining the variation in the total number of dogs (n) in the various analyses described below.

4.2. Heart murmur

All murmur grades (using both the 1-6 scale and the mild-severe with precordial thrill scale) and murmur intensities (very soft-extremely loud) were represented. Murmur characteristics are presented in *Table 1*.

***Table 1**. Information about the murmur characteristics of the 53 dogs included in the study. Murmur grades 1 and 2 were combined for statistical analysis as there were only two murmurs graded 1/6. The total number of dogs (n) varies for different murmur characteristics as some characteristics were not obtainable in some of the dogs (for instance, if there were a lot of respiratory sounds while listening close to the chest wall, it was not possible to determine if the murmur was audible or not). Proportion of dogs out of the total number of dogs for each characteristic is shown within brackets.*

Characteristic	Total number of dogs (n), number of dogs, [percent, %]
Murmur grade	n = 53
1+2	2+9 [21%]
3	12 [23%]
4	13 [25%]
5	11 [21%]
6	6 [11%]
Murmur grade	n = 53
Mild	11 [21%]
Moderate	12 [23%]
Severe without precordial thrill	13 [25%]
Severe with precordial thrill	17 [32%]
Murmur intensity	n = 53
Very soft	2 [4%]
Soft	9 [17%]
Moderate	12 [23%]
Loud	14 [26%]
Very loud	12 [23%]
Extremely loud	4 [8%]
Precordial thrill	n = 50
Yes	17 [34%]
No	33 [66%]
Audible when listening with ear over PMI*	n = 44
Yes	32 [73%]
No	12 [27%]

Characteristic	Total number of dogs (n), number of dogs, [percent, %]
Audible with chest piece lifted slightly	n = 48
Yes	11 [23%]
No	37 [77%]
Murmur PMI*	n = 53
Mitral valve	51 [96%]
Tricuspid valve	2 [4%]
Murmur localization and radiation	n = 50
All valve areas	45 [90%]
Only mitral valve	4 [8%]
Only tricuspid valve	1 [2%]

**PMI = point of maximum intensity.*

The descriptive murmur intensity did not match the numerical murmur grade in three dogs. Two grade 6 murmurs were described as ‘‘very loud’’, and one grade 5 murmur was described as ‘‘loud’’.

4.2.1. Dog characteristics and echocardiographic findings in dogs with certain heart murmur characteristics

In the group of murmurs that could be heard when listening close to the chest wall (without the use of a stethoscope), murmur grades 2-6 were represented. Murmur grades 2, 3, 4, 5, and 6 were represented by 1, 8, 8, 10, and 5 dogs, respectively. In these dogs BCS 3-7 were represented, the dogs were in ACVIM stages B1-D, and their MR jets had a lateral, central, or septal direction. The median heart rate at auscultation was 124 BPM (IQR 120-140). All chest shapes (normal, deep, or round) were represented. Furthermore, in the group of dogs with murmurs that could not be heard without stethoscope murmur grades 1, 2, 3, and 4 were represented by 2, 6, 3, and 1 dog/s.

Three grade 3 murmurs and two grade 4 murmurs could be auscultated with the chest piece slightly lifted off the chest wall. In these dogs BCS 4-6 were represented, the dogs were in ACVIM stages B1-C, and their jets were lateral, central, or septal. The median heart rate at auscultation was 120 BPM (IQR 110-142). The chest shapes normal and round were represented.

4.3. Echocardiography

The median heart rate during echocardiography (n = 53) was 130 BPM (IQR 110-150). The median LVIDDN (n=53) was 1.8 (IQR 1.6-2.1), the median LA/Ao was 1.6 (IQR 1.2-1.7), and the median LVIDSN was 1.1 (IQR 1.0-1.3). Furthermore, the median FS (n=52) was 40.9 % (IQR 34.6-45.0).

4.3.1. Valve regurgitation

Twenty-five percent of the dogs (total n=53) had mild MR (n=13), 5.7% had mild to moderate MR (n=3), 36% had moderate MR (n=19), 9.4% had moderate to severe MR (n=5), and 25% had severe MR (n=13). Sixty-five percent of dogs (total n = 50) had a lateral jet (n=33), followed by 22% with a central jet (n=11) and 12% with a septal jet (n=6). One dog had two jets (lateral and septal). The median MR velocity (n=48) was 6.0 m/s (IQR 5.4-6.2), and the median angle of the MR jet (n=48) was 68.2 degrees (IQR 57.0-92.8).

In 4/53 dogs (7.5%) mild aortic regurgitation was seen, and 4/53 dogs (7.5%) had mild pulmonic regurgitation. Seventy-four percent (n=39/53) of dogs had some degree of tricuspid regurgitation (TR). Mild TR was seen in 68% (n=36) of all dogs, while mild to moderate TR was seen in one dog and moderate TR in two dogs. Furthermore, the median TR velocity (n=31) was 2.8 m/s (IQR 2.4-3.2). A total of 12 dogs (35%) had TR ≥3, which is suggestive of pulmonary hypertension.

4.4. Association with ACVIM stage

4.4.1. Dog characteristics

Dog characteristics for the dogs classified into the various ACVIM stages are presented in *Table 2*. No statistical difference was found between ACVIM stages for the various dog characteristics, and no statistical analysis could be performed for chest shape, why no p-value is presented for this characteristic.

4.4.2. Heart murmur

None of the dogs in stage B1 had murmurs graded 5-6/6. None of the dogs in stage B2 had grade 1-2/6 murmurs, and none of the dogs in stages C-D had grade 1-3/6 murmurs. Furthermore, none of the stage B1 dogs had a murmur classified as severe with a precordial thrill, none of the dogs in stage B2 had mild murmurs, and none of the dogs in stages C-D had mild or moderate murmurs. Regarding murmur intensity, none of the dogs in stage B1 had very loud or extremely loud murmurs. None of the dogs in stage B2 had very soft or soft murmurs, and none of the dogs in stages C-D had very soft, soft, or moderate murmurs. See *Figure 2* for more information.

None of the dogs in stage B1 had a precordial thrill, whereas 43% and 78% of dogs in stage B2 and stages C-D, respectively, had precordial thrills. A statistical difference was seen between B1 and B2, as well as B1 and C-D. Moreover, the murmur could be heard when listening close to the chest wall (without a stethoscope) in 44%, 86%, and 100% of the dogs in stages B1, B2, and C-D, respectively. Moreover, the murmur was audible when the chest piece was lifted slightly off the chest wall in 11%, 18%, and 63% of dogs in stages B1, B2, and C-D, respectively. For further information, see *Figure 3*.

4.4.3. Echocardiography

The echocardiographic variables LVIDDN, LA/Ao, and LVIDSN are presented in *Table 2*. The heart rate at echocardiography was higher in stages C-D, compared to both stage B1 and stage B2. See *Table 2*.

All dogs in stages C-D had severe MR, dogs in stage B2 had mild-severe MR, and dogs in stage B1 only had mild-moderate MR (see *Table 2*). A majority (65% and 83%, respectively) of the dogs in stages B1 and B2 had a jet with a lateral direction, while 60% of dogs in stages C-D had a central jet. See *Figure 4*.

The MR velocity was higher in stage B2, compared to stages C-D. The angle of the MR jet was greater in stages C-D, compared to stage B2. See *Table 2*.

All the dogs in stage B1 and all dogs except one in stage B2 that had TR, had mild TR. The two dogs with moderate TR were in stages C-D. The TR velocity did not statistically differ between stages. See *Table 2* for more information.

Table 2. *Summary of dog characteristics, clinical, and echocardiographic data in the 53 recruited dogs by stage of disease severity based on the ACVIM (American College of Veterinary Internal Medicine) classification. The total number of dogs varies for different factors as some factors were not obtainable in some of the dogs. Data are presented as median and (interquartile range), or absolute number and [percent, %]. The percent is the percent of total dogs for the specific variable investigated. Some categorical data are presented descriptively because no reliable statistical analysis could be performed. The probability>ChiSquare value for the likelihood ratio test is presented for the categorical data BCS (body condition score), CKCS (Cavalier King Charles Spaniel), and sex. For numerical data, the probability>ChiSquare value is presented. If this value was <0.05, further analysis was performed and in those cases three additional p-values are presented. For the latter p-values, a Bonferroni adjusted p-value of 0.017 was used for statistical significance.*

Factor	**Total number of dogs**	**Stage B1**	**Stage B2**	**Stages C+D**	**P-value**
Body weight (kg)	53	8.4 (5.2-9.9)	9.2 (6.4-11.4)	6.3 (3.8-8.4)	0.1665
BCS – normal/overweight	51	14/5 [27/10]	12/12 [24/24]	7/1 [14/2]	0.0811
Age at examination (years)	52	9.2 (7.8-11.2)	11.6 (8.7-13.4)	11.9 (9.4-13.0)	0.0505
CKCS - yes/no	53	8/11 [15/21]	8/16 [15/30]	3/7 [6/13]	0.7650
Sex - female/male	53	8/11 [15/21]	5/19 [9/36]	3/7 [6/13]	0.3205
Chest shape - deep/normal/round	53	8/3/8 [15/6/15]	5/8/11 [9/15/21]	1/5/4 [2/9/8]	-
HR at auscultation (BPM)	53	124.0 (108.0-140.0)	120.0 (108.5-137.5)	134.0 (120.0-160.0)	0.1232
HR at echocardiography (BPM)	50	120.0 (110.0-140.0)	127.5 (111.3-130.0)	158.0 (138.8-170.0)	0.0058 0.0016 (C-B2) 0.0092 (C-B1) 0.8824 (B2-B1)

Factor	Total number of dogs	Stage B1	Stage B2	Stages C+D	P-value
PMI - mitral valve/tricuspid valve	53	18/1 [34/2]	24/0 [45/0]	9/1 [17/2]	-
Murmur radiation – all valve areas/only mitral valve/only tricuspid valve	50	13/4/1 [26/8/2]	23/0/0 [46/0/0]	9/0/0 [18/0/0]	-
LVIDDN	53	1.6 (1.5-1.7)	1.9 (1.7-2.1)	2.5 (2.3-2.6)	<.0001 <.0001 (C-B1) 0.0002 (C-B2) 0.0004 (B2-B1)
LA/Ao	53	1.17 (1.1-1.2)	1.6 (1.6 -1.7)	2.2 (1.9-2.6)	<.0001 <.0001 (B2-B1) <.0001 (C-B1) 0.0001 (C-B2)
LVIDSN	53	1.0 (0.9-1.1)	1.11 (1.0-1.2)	1.5 (1.3-1.5)	0.0009 0.0028 (C-B2) 0.0008 (C-B1) 0.1323 (B2-B1)
FS (%)	52	37.6 (31.0-44.9)	41.8 (36.2-45.7)	41.5 (38.9-46.0)	0.2028

Factor	Total number of dogs	Stage B1	Stage B2	Stages C+D	P-value
MR – mild/mild to moderate/moderate/moderate to severe/severe	53	12/3/4/0/0 [23/6/8/0/0]	1/0/15/5/3 [2/0/28/9/6]	0/0/0/0/10 [0/0/0/0/19]	-
MR – velocity (m/s)	47	5.9 (5.3-6.2)	6.1 (5.7-6.4)	5.2 (4.7-6.0)	0.0162 0.2267 (B2-B1) 0.1187 (C-B1) 0.0047 (C-B2)
MR – direction (degrees)	48	82.0 (45.3-94.5)	65.0 (53.5-70.5)	90.0 (81.2-96.3)	0.0330 0.0056 (C-B2) 0.1531 (C-B1) 0.3795 (B2-B1)
TR – mild/mild to moderate/moderate	39	10/0/0 [26/0/0]	18/1/0 [46/3/0]	8/0/2 [21/0/5]	-
TR – velocity (m/s)	31	2.5 (2.1-3.0)	2.8 (2.5-2.9)	3.3 (2.8-3.8)	0.0361 0.0193 (C-B1) 0.0546 (C-B2) 0.4748 (B2-B1)

BCS = body condition score. CKCS = Cavalier King Charles Spaniel. HR = heart rate. BPM = beats per minute. PMI = point of maximum intensity. LVIDDN = normalized left ventricular internal diameter in diastole. LA/Ao = left atrial-to-aortic ratio. LVIDSN = normalized left ventricular internal diameter in systole. FS = fractional shortening. MR = mitral regurgitation. TR = tricuspid regurgitation.

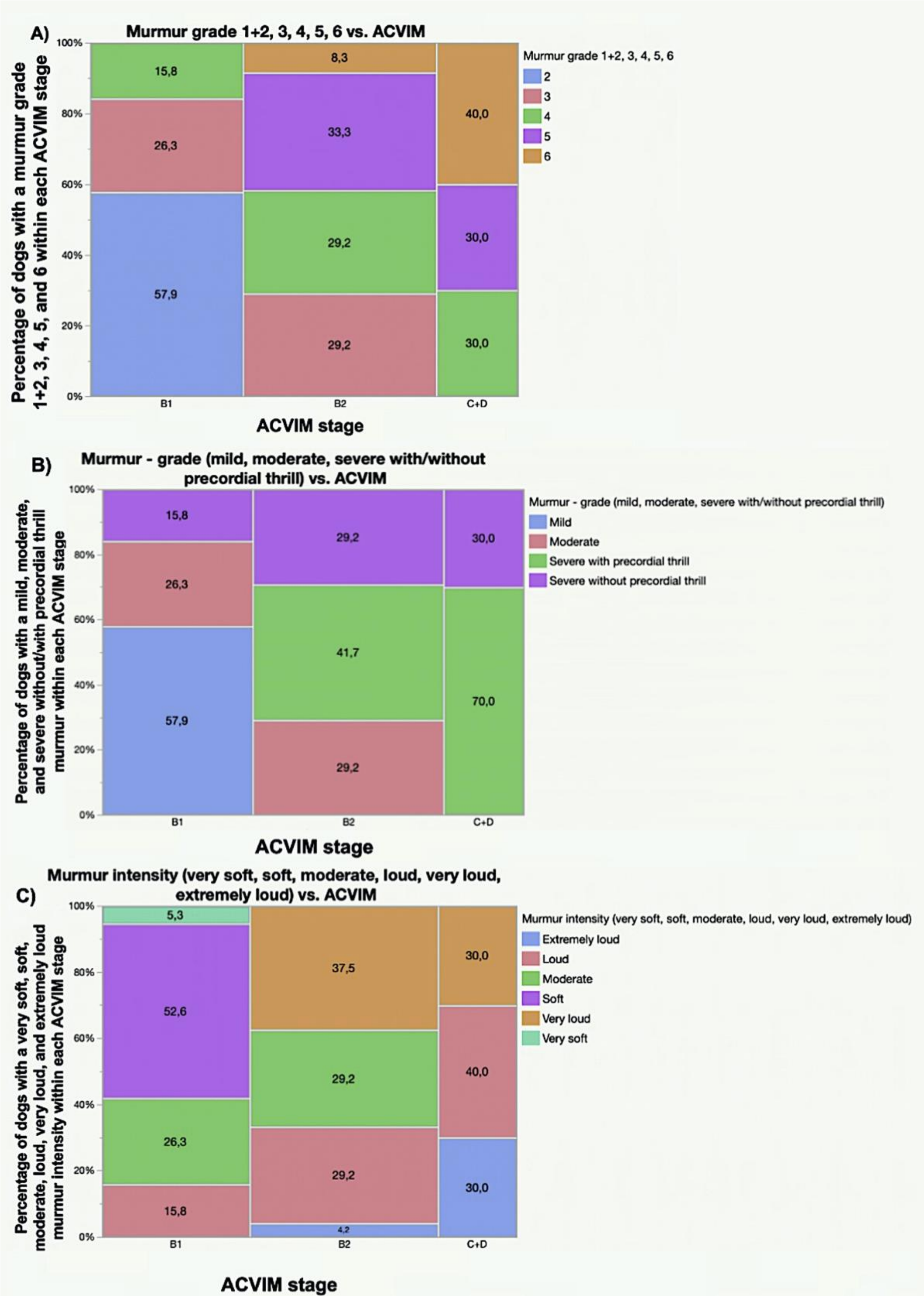

Figure 2.
A) *Distribution of 53 dogs with different murmur grades (1+2-6) by American College of Veterinary Internal Medicine (ACVIM) stages: 19 (B1), 24 (B2), and 10 (C-D).*
B) *Distribution of 53 dogs with different murmur grades (mild-severe with precordial thrill) by ACVIM stages: 19 (B1), 24 (B2), and 10 (C-D).*
C) *Distribution of 53 dogs with different murmur intensities (soft-extremely loud) by ACVIM stages: 19 (B1), 24 (B2), and 10 (C-D). No statistical analysis using Chi-square tests could be performed because the basic assumptions of this test were not fulfilled.*

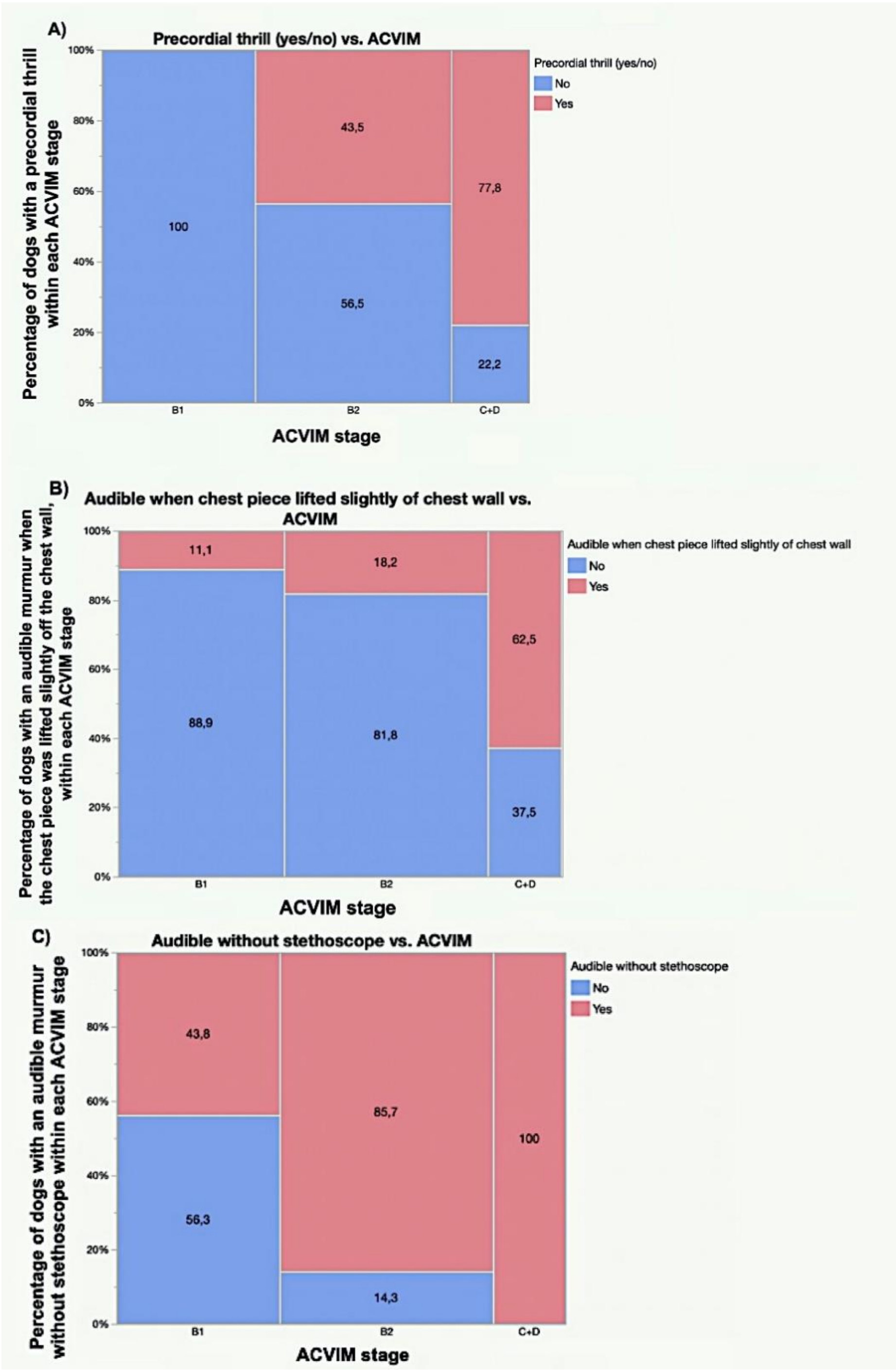

Figure 3.
A) *Distribution of 50 dogs with and without a precordial thrill by American College of Veterinary Internal Medicine (ACVIM) stages: 18 (B1), 23 (B2), and 9 (C-D).*
B) *Distribution of 48 dogs with and without an audible murmur when the chest piece was lifted slightly off the chest wall by ACVIM stages: 18 (B1), 22 (B2), and 8 (C-D).*
C) *Distribution of 44 dogs with and without an audible murmur when listening close to the chest wall without a stethoscope by ACVIM stages: 16 (B1), 21 (B2), and 7 (C-D).*

A statistical difference was seen for precordial thrill by ACVIM between B1 and B2 (p-value: 0.0021), as well as B1 and C-D (p-value: <.0001), using the 2-tailed Fisher's exact test. A Bonferroni adjusted p-value of 0.017 was used for statistical significance. No statistical difference was seen between B2 and C-D. No statistical analysis using Chi-square tests could be performed for the remaining factors because the basic assumptions of this test were not fulfilled.

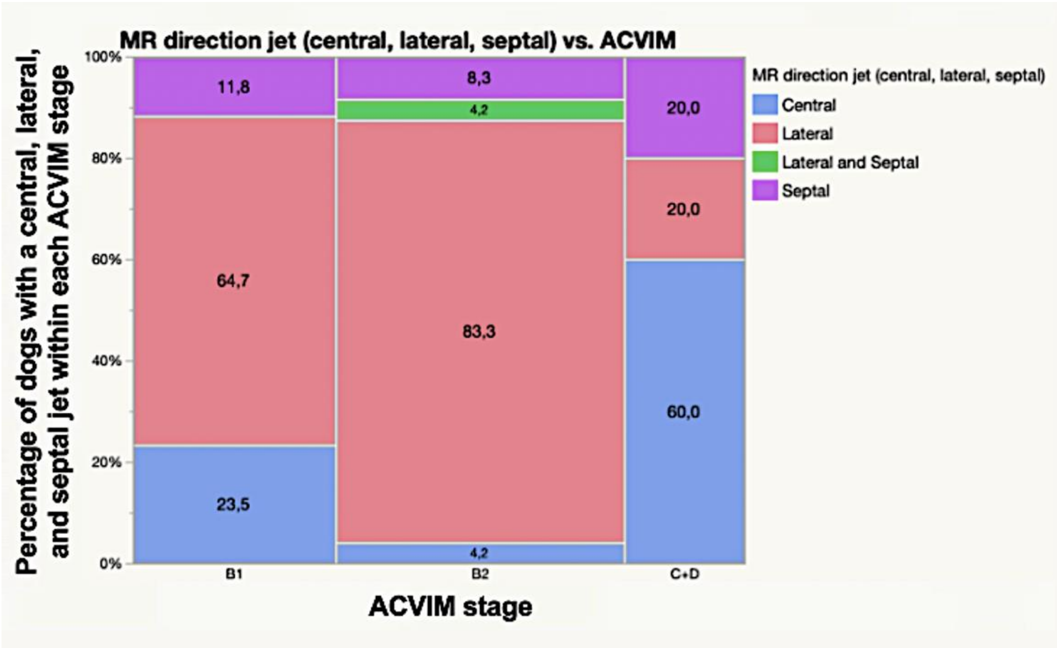

Figure 4. *Distribution of the direction of the mitral regurgitation (MR) jet in 51 dogs by American College of Veterinary Internal Medicine (ACVIM) stages: 17 (B1), 24 (B2), and 10 (C-D). One dog had two jets (lateral and septal) in stage B2. No statistical analysis using Chi-square tests could be performed because the basic assumptions of this test were not fulfilled.*

4.5. Direction of the MR jet

The angle of the MR jet was compared to the murmur grade (mild-severe with precordial thrill). See *Table 3*.

Table 3. *The median and (interquartile range) of the angle, in degrees, of the mitral valve regurgitation jet in relation to the mitral valve orifice plane, by different murmur grades in 48 dogs. No statistical difference was found between the murmur grades.*

Murmur grade	Angle in degrees – median (IQR)
Mild	82.0 (67.2-113.5)
Moderate	67.0 (49.0-97.0)
Severe without precordial thrill	66.0 (38.0-96.0)
Severe with precordial thrill	77.4 (56.0-91.5)

5. Discussion

The present study aimed to evaluate potential factors that might impact murmur assessment, as well as to evaluate the utility of various murmur grading scales in dogs with MMVD. In the present study, several murmurs of various grades could be heard when listening with the ear close to the chest wall, and some also when the chest piece of the stethoscope was lifted slightly off the chest wall. Approximately 43% of dogs in stage B2 had a palpable precordial thrill. The majority of the dogs in preclinical MMVD had a jet with a lateral direction, while 60% of the dogs in stages C-D had a central jet. The descriptive murmur intensity matched the numerical murmur grade in all dogs except three. The murmur grade increased with ACVIM stage.

The present study found that numerous murmurs of various grades could be heard when listening close to the chest wall (without a stethoscope). A few murmurs that were graded <6/6, remained audible when the chest piece was lifted slightly off the chest wall. In the reviewed murmur grading scales, only a grade 6 murmur is defined as remaining audible when the chest piece is lifted slightly off the chest wall (Kvart & Häggström 2002; Smith *et al*. 2006; Prošek 2017; Ware & Ward 2020b). Furthermore, to be able to hear the murmur when listening with the ear close to the chest wall is mentioned as a criterion for grade 6 murmurs in one of the 6-level murmur grading scales reviewed (Smith *et al*. 2006), but not mentioned at all in the other 6-level murmur grading scales reviewed (Kvart & Häggström 2002; Prošek 2017; Ware & Ward 2020b). Potential explanations for these findings in our study could not be thoroughly investigated in the population of recruited dogs. Nonetheless, reasons for increased loudness of all heart sounds include a thin chest and enhanced ventricular contraction (Smith *et al*. 2006). However, none of these factors was a common denominator in the dogs where the murmur remained audible when the chest piece was lifted slightly of the chest wall, or in the dogs where the murmur was audible when listening close to the chest wall without a stethoscope in this study.

In the present study, a lateral jet direction was the most common in dogs with preclinical disease, whereas a central jet direction was the most common in stages C-D. However, the study group was relatively small, and only 10 dogs were included in stages C-D. Furthermore, the present study found that the numerical angle of the

MR jet was statistically higher in stages C-D, compared to stage B2. Ljungvall and Häggström (2017) state that dogs with MMVD often have an MR jet with a lateral direction. This is thought to be because the anterior leaflet of the mitral valve is longer and, therefore, more mobile compared to the posterior leaflet in both dogs and humans (Ahmed *et al*. 2009; Borgarelli 2004 see Ljungvall & Häggström 2017). The anterior leaflet, therefore, has a greater risk of prolapsing, compared to the posterior leaflet (Ljungvall & Häggström 2017). A higher prevalence of prolapse of the anterior leaflet in dogs has been seen in a study (Terzo *et al*. 2009). The results in the present study indicate that the jet starts with a lateral direction and becomes more central in more advanced disease. This could be explained by worsening MR in more advanced disease due to dilatation of the ventricle, causing separation of the mitral leaflets (Ware & Ward 2020a). Furthermore, this could explain why some dogs with mild disease on the echocardiogram sometimes have a higher-intensity murmur, as seen in the study by Ljungvall *et al.* (2014). A lateral jet is closer to the auscultation area, and, as previously mentioned, the distance the sound waves have to travel affects the intensity (Smith *et al*. 2006; Ahlström 2008). Furthermore, Prošek (2017) suggests that the direction of the jet influences the murmur intensity at the chest wall. However, this is simplified, and there are certainly more factors to consider.

None of the dogs in stage B1 had a precordial thrill, whereas 43% and 78% of dogs in stage B2 and stages C-D, respectively, had precordial thrills. This percentage of dogs with precordial thrills in stage B2 might appear high. This result is, however, similar to the result in the study by Ljungvall *et al.* (2014), which had a larger group of dogs included (total n=578). In this study a few percent of dogs in stage B1 had thrilling murmurs, slightly less than 30% of dogs in stage B2 had thrilling murmurs, and about 60% of dogs in stages C-D had thrilling murmurs. However, the study by Ljungvall *et al.* (2014) was retrospective, whereas this study was prospective. Additionally, some thrills are very pronounced, but some are not. The less pronounced thrills might be classified as thrills by some observers, whereas others do not classify them as thrills. This might, therefore, have impacted the result of the present study, and it might also impact murmur grading in the routine clinic. As previously mentioned, all dogs that had a precordial thrill (in other words, dogs with murmurs graded 5/6 or 6/6) were in stage B2 or higher. This indicates that having a murmur with a precordial thrill is associated with more advanced disease and that medical treatment with pimobendan likely is indicated. This result is also in agreement with previous studies that have shown an association between murmur grade and MMVD severity (Häggström *et al*. 1995; Pedersen *et al*. 1999; Kvart *et al*. 2002; Olsen *et al*. 2003; Soares *et al*. 2005; Ljungvall *et al*. 2009, 2014; Lopez-Alvarez *et al*. 2015; Franchini *et al*. 2021; Wilshaw *et al*. 2021).

The murmur intensity scale (very soft-extremely loud) matched the murmur grades 1-6 in all murmurs except three. Because there were only a few exceptions in the investigated group of dogs, a potential explanation for this finding could not be thoroughly investigated in the population of recruited dogs. The intensity scale (very soft-extremely loud) used was basically the same as the intensities presented in the murmur grading scale by Smith *et al*. (2006) (the present study used the description ‘‘moderate’’ instead of the description ‘‘louder’’). Kvart and Häggström (2002) defined their murmur intensities as low (grades 1-2), moderate (grade 3), and high (grades 4-6). Ware and Ward (2020b) and Prošek (2017) defined the intensities accordingly: very soft (grade 1), soft (grade 2), moderate (grade 3), loud (grades 4-5), and very loud (grade 6). In the present study, two grade 6 murmurs were described as ‘‘very loud’’, and one grade 5 murmur was described as ‘‘loud’’. The previously mentioned intensities that did not match the murmur grading scale by Smith *et al.* (2006) did, however, match the murmur grading scales by Prošek, as well as by Ware and Ward.

For most dog characteristics (body weight, age at examination, HR at auscultation, BCS, CKCS – yes/no, sex), no statistical difference was found between ACVIM stages in this study. No statistical analysis using Chi-square tests could be performed for chest shape because the basic assumptions of this test were not fulfilled. However, body weight and BCS could potentially affect murmur grading as obesity decrease loudness of all heart sounds (Smith *et al*. 2006). The body weight was lower for dogs in stages C-D compared to other stages, but a statistical difference could not be found. Only dogs with BCS 4-7 were represented in statistical analysis. Therefore, further research, including a larger study group, is needed to further evaluate if body weight and BCS have any impact on murmur grading.

Chest shape could also potentially impact murmur grading. Smith *et al.* (2006), as mentioned before, list a thin chest as a reason for increased heart sounds. Theoretically, different chest shapes result in different distances between the heart and the auscultation area, and the distance the sound has to travel from origin to the listener affects the intensity of the murmur (Smith *et al*. 2006; Ahlström 2008). However, more research with larger study groups is needed to evaluate this properly.

In the present study, the breeds were divided into two groups: CKCS and other breeds. No statistical difference between ACVIM stages was found in this study. However, many breeds were combined into one group, which might have impacted the result. Previous studies have not identified an impact of breed on the agreement between murmur intensity and echocardiographic disease severity (Caivano *et al*. 2018; Rishniw *et al*. 2019). However, these studies evaluated dogs with subaortic and pulmonic stenosis and not MMVD. It is important to note that breed is a factor that indirectly ‘‘includes’’ other factors that might be the primary affecting factor on murmur assessment. For instance, the chest shape might be the same for dogs in a certain breed.

In this study, no significant difference was found between dogs in various ACVIM stages concerning heart rate at auscultation. Furthermore, an elevated heart rate at auscultation was not a common denominator in dogs where the murmur remained audible when the chest piece was lifted slightly of the chest wall, or in the dogs where the murmur was audible when listening close to the chest wall without a stethoscope. However, previous studies have shown that low-intensity murmurs can increase in intensity due to stress and that stress can reveal a murmur not heard in a non-stressed animal (Pedersen *et al*. 1999; Höglund *et al*. 2004).

In the present study, the murmur grade for both the 6-level scale and the 4-level scale (mild, moderate, severe without precordial thrill and severe with precordial thrill) showed a pattern of increasing with the ACVIM stage of the disease (no statistical analysis performed). This is in agreement with previous studies that have shown an association between murmur grade and severity of MMVD (Häggström *et al*. 1995; Pedersen *et al*. 1999; Kvart *et al*. 2002; Olsen *et al*. 2003; Soares *et al*. 2005; Ljungvall *et al*. 2009, 2014; Lopez-Alvarez *et al*. 2015; Franchini *et al*. 2021; Wilshaw *et al*. 2021). In the present study, a majority (58%) of dogs in stage B1 had murmurs graded 1-2 (none of the dogs in stage B1 had a murmur accompanied by a precordial thrill), whereas a majority (70%) of dogs in stages C-D had murmurs graded 5-6. In stage B2, the percentage of dogs with murmurs 3, 4, and 5 were about the same (respectively), as well as a few percent with grade 6 murmurs. This is in agreement with the study by Ljungvall *et al.* (2014), where the authors concluded that soft murmurs (grades 1-2) indicate mild disease, while thrilling murmurs (grades 5-6) indicate more advanced disease. They also concluded that no accurate conclusions about disease severity can be made for grade 3 and grade 4 murmurs from auscultation alone. Therefore, it might be argued that both the 4-level and 6-level murmur grading scales are useful for MMVD dogs as they can be an indicator of disease severity and disease progression. However, more research is needed because there are exceptions where murmur grade and disease severity (based on echocardiographic examination) are not in agreement.

The results of this study indicate that certain criteria in murmur grading scales might be redundant. As previously discussed, the murmur intensities matched some of the murmur grading scales reviewed, but not others, and the murmur could be heard without a stethoscope for several murmur grades. Only a few studies have investigated the clinical relevance of various murmur grading scales. Previous studies have shown that no essential clinical information is lost when using a 4-level scale, compared to using a 6-level scale for dogs with MMVD as well as for dogs with pulmonic or subaortic stenosis (Ljungvall *et al*. 2014; Caivano *et al*. 2018). The fact that there have not been many studies evaluating the clinical relevance of murmur grading scales might explain why the 6-level murmur grading scales reviewed for this degree project were not identical (Kvart & Häggström 2002; Smith *et al*. 2006; Prošek 2017; Ware & Ward 2020b). This is also in agreement with the study by Keren *et al.* (2005), where none of the participants defined grades 1-3/6 precisely like Levine (this was a study in humans, not dogs). It might be argued that a scale with fewer and less specific grades would be less complicated and easier to use than a 6-level scale. This is partly supported by the study by Keren *et al.* (2005), where grading accuracy and consistency improved when using the internal reference system (3-level scale) for some groups of participants in the study and some murmur grades. However, no statistically significant improvement in interobserver agreement was seen in that study. Diagnostic imaging is usually carried out when a murmur is discovered. It might be argued that it, therefore, does not matter if the murmur grade is ‘‘wrong’’ or if we do not define our murmur grades the same, as the results from echocardiography matter more. However, it could matter in cases where murmur grading is used to monitor the disease progression, for instance, in a dog with a previously diagnosed mild murmur that has been given a diagnosis of mild MMVD based on echocardiographic examination results. These dogs might be recommended to undergo a new echocardiographic examination if the murmur increases in intensity. However, with various definitions of the murmur grades, there is a risk of grading murmurs incorrectly or differently compared to other veterinarians. Therefore, the follow-up echocardiographic examination might be delayed (which would delay initiation of treatment) in some dogs if murmurs are graded lower than they indeed are. Further studies are needed to evaluate the clinical relevance of the various murmur grading scales (both regarding how many levels are clinically necessary and which criteria the various definitions ideally should consist of).

It might also be argued that a murmur grading scale with fewer grades is easier for inexperienced veterinarians to use. This aspect was not investigated in the present study. However, when, for instance, the descriptive murmur intensity does not match the grading scale one uses (as seen in this study), there is a risk of grading murmurs incorrectly. Additionally, many veterinary students, who have only listened to a few murmurs, find the 6-level scale difficult to learn and understand. In the study by Pedersen *et al.* (1999), the more experienced observers’ murmur gradings (the 6-level scale was used) correlated with the size of the jet, while the two least experienced observers’ gradings did not. Murmur grading requires practice no matter what scale is used, but it would be interesting to investigate whether a 4-level scale has better interobserver agreement than a 6-level scale. However, as mentioned above, no statistically significant improvement in interobserver agreement was found for the 3-level scale evaluated by Keren *et al. (2005).*

5.1. Limitations

The limited study time resulted in a relatively small study group, making it more difficult to draw firm conclusions.

Another factor that might have impacted the study is that most factors studied and collected were subjectively assessed. There are, however, ways to improve reliability and limit bias. For instance, several people could have auscultated all dogs, or the murmurs could have been recorded and evaluated by several people. Moreover, the study was not blinded, and assessments could be biased by knowledge of, for instance, medical history.

Several people were involved in the assessments, which have both advantages and disadvantages. With several people, there is potential interobserver variability. However, having more people involved, compared to only one person, can limit bias. Furthermore, different stethoscopes were used by the supervisors and the student. However, all murmur characteristics assessed by the student were approved by a supervisor.

The most common drugs among the dogs in this study were pimobendan (32%) and furosemide (17%). Due to the small study group, no conclusions about if the treatment protocol affects murmur grading could be made. However, the positive inotropic effect of pimobendan (FASS n.d.) might have influenced the murmur intensity. However, more research is needed to investigate a potential effect.

5.2. Future studies

Further research is needed on this topic. Data collection has continued after the present study was finished, and the ongoing study will include a more extensive study group. Furthermore, additional statistical analyses will be performed, which hopefully will provide more knowledge about the potential factors that might impact murmur grading, as well as more insight about the utility of various murmur grading scales.

Additional factors that might affect murmur grading could be investigated in future studies, such as the quality of the murmur and fur length. Furthermore, one interesting perspective to investigate in future studies would be to compare the murmur grading of, for instance, students and more experienced veterinarians. This could be done for several murmur scales, including the 6-level murmur scale, the 4-level murmur scale, and the 3-level murmur scale.

5.3. Conclusions

In summary, several murmurs could be heard when listening with the ear close to the chest wall, as well as some when the chest piece of the stethoscope was lifted slightly off the chest wall. Many dogs in stage B2 had a palpable precordial thrill. A lateral jet direction was more common in preclinical MMVD, whereas a central jet was more common in stages C-D. In a few cases the descriptive murmur intensity did not match the numerical murmur grade. The murmur grade increased with ACVIM stage for both the 4-level and 6-level murmur grading scales.

In conclusion, whether the murmur can be heard without a stethoscope and specific intensity descriptions for each grade might be redundant criteria in murmur grading scales. Both the 4-level and 6-level scales might be useful in dogs with MMVD, but more research is needed to further evaluate the utility of these and other scales. Furthermore, no firm conclusions about if the factors investigated impact the agreement between murmur grading and disease severity can be drawn from the results in this study. Factors such as BCS, murmur characteristics, and the direction of the MR jet are still considered potential factors that could impact murmur grading, but further research with a larger study population is needed.

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Popular Science Summary

Myxomatous mitral valve disease (MMVD) is the most common heart disease in dogs. In a dog with MMVD, the heart valve between the left atrium and chamber (this is the valve that is most often affected, although all heart valves can be affected) becomes thickened and, therefore, it is not able to close like it is supposed to. This means that blood will leak in the wrong direction (from the left chamber to the left atrium). The disease gets worse with time, and eventually, the heart will become enlarged. The heart can eventually start to fail, and the dog will develop clinical signs, such as difficulty breathing. However, this does not happen in all dogs.

The blood that leaks in the wrong direction through the diseased valve causes a heart murmur (an abnormal sound) that a veterinarian can hear when listening to the dog's heart. The murmur can arise many years before the dog develops noticeable clinical signs caused by the disease. When a murmur is detected, and the veterinarian suspects that the dog has MMVD, echocardiography (ultrasonographic examination of the heart) is usually recommended as the next step. Not only can the diagnosis be confirmed, the severity of the disease can also be assessed. In dogs where the heart has become enlarged to a certain degree, medical treatment is indicated, which can prolong the preclinical period (time without clinical signs).

Veterinarians usually grade heart murmurs according to a murmur grading scale that consists of 6 grades. A grade 1 murmur has the lowest intensity, and a grade 6 murmur has the highest intensity. In several studies, researchers have found an agreement between murmur grade and disease severity on echocardiography (meaning that a grade 1 murmur reflects mild disease, while a grade 6 murmur reflects severe disease). This agreement between murmur grade and disease severity is, however, not complete.

In this study we aimed to evaluate potential factors that might impact murmur assessment and to evaluate the utility of various murmur grading scales in a group of dogs with MMVD. Evaluation of the utility of various murmur grading scales could potentially help optimize existing murmur grading scales. Moreover, knowledge about factors impacting murmur grading could potentially lead to a better understanding of the association between murmur grades and disease severity (based on echocardiographic examination).

This study included 53 dogs, all diagnosed with a heart murmur, that were scheduled for cardiac examination at the Cardiology Clinic at the University Animal Hospital at Swedish University of Agricultural Sciences (SLU) in Uppsala, Sweden. The dogs were included if they received a diagnosis of MMVD after an echocardiographic examination. Information about dog characteristics (such as age and body weight), data from clinical examination (such as murmur grade), and echocardiographic findings (such as different measurements of the size of the heart) were collected.

Results showed that the heart murmur could be heard when listening with the ear (without the use of a stethoscope) close to the chest wall in 73% of dogs. The percent of dogs in which the murmur was hearable when listening close to the chest wall increased with increasing disease severity. When listening with a stethoscope, the murmurs remained audible when the stethoscope was lifted slightly off the chest wall in 23% of total dogs. Three grade 3 murmurs and two grade 4 murmurs remained audible with the chest piece lifted slightly off the chest wall. According to the reviewed murmur scales, only grade 6 murmurs are defined as being audible with the stethoscope lifted off the chest wall. A precordial thrill (a vibration that can be felt when placing a hand over the chest wall) was palpable (felt) in 34% of dogs. The majority of dogs with mild MMVD had a laterally directed jet (this means that the blood that leaks from the chamber to the atrium has a direction towards the chest wall of the dog), while it was central (the blood leaks ‘‘straight up’’ into the atrium) in 60% of the dogs with more severe disease. The murmur grade increased with increasing disease severity.

In conclusion, whether the murmur can be heard without a stethoscope and specific intensity descriptions for each grade might be redundant criteria in murmur grading scales. Both the 4-level and 6-level scales might be useful in dogs with MMVD, but more research is needed to further evaluate the utility of these and other scales. Furthermore, no firm conclusions about if the factors investigated impact the agreement between murmur grading and disease severity (based on echocardiographic assessment) can be drawn from the results in this study. Factors such as body condition score, murmur characteristics, and the direction of the MR are still considered potential factors that could impact murmur grading, but further research with a larger study population is needed.