UNIVERSIDADE DE LISBOA

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COMPARISON OF HEART MEASUREMENTS IN THORACIC RADIOGRAPHS BEFORE AND AFTER THE TREATMENT OF PULMONARY EDEMA IN DOGS WITH DEGENERATIVE MITRAL VALVE DISEASE: A RETROSPECTIVE STUDY OF 18 CLINICAL CASES

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DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

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Resumo

Comparação de medições cardíacas em radiografias torácicas antes e depois do tratamento de edema pulmonar em animais com Doença Degenerativa da Válvula Mitral: um estudo retrospetivo de 18 casos clínicos

A Doença Degenerativa da Válvula Mitral (DDVM) tem a prevalência mais alta de todas as doenças cardíacas caninas, representando 75-80% dos casos destes doentes.

A DDVM é caracterizada pela sua natureza evolutiva. Assim à medida que a doença progride, as alterações microscópicas e macroscópicas da válvula mitral tornam-se mais graves e começam gradualmente a impedir o seu normal funcionamento. Uma das complicações que pode ocorrer é o desenvolvimento de edema pulmonar que sucede quando a capacidade do sistema linfático do pulmão é excedida, levando, por isso, à acumulação de conteúdo aquoso no compartimento extravascular dos mesmos.

A etiologia e consequentemente a cura da DDVM não são atualmente conhecidas, dai a importância em perceber e desenvolver ferramentas que permitam a monitorização da doença. Embora a melhor maneira de determinar e confirmar o diagnóstico de DDVM seja através de uma ecocardiografia, este exame de diagnóstico representa um investimento para o proprietário, necessita de material caro e exige um nível de competência mais elevado para o realizar e interpretar. Simultaneamente, a realização de radiografias do tórax é uma técnica amplamente disponível e económica, o que justifica o interesse em estudar a evolução das medidas radiográficas *Vertebral Heart Score* (VHS) e *Vertebral Left Atrium Size* (VLAS) em cães com DDVM.

O objetivo deste estudo retrospetivo prende-se com a comparação de medidas cardíacas, em radiografias da cavidade torácica, antes e depois do tratamento de edema pulmonar em 18 cães com DDVM que foram apresentados em consulta num centro hospitalar veterinário de referência francês.

A principal conclusão deste estudo indica que o tamanho do átrio esquerdo e da silhueta cardíaca diminui depois da resolução do edema pulmonar de origem cardíaca, quando comparado com as dimensões durante a sua ocorrência. Adicionalmente, esta diminuição de tamanho do átrio esquerdo é detetável utilizando o método VLAS, o que confirma o seu valor na monitorização da progressão da doença. Consequentemente, é possível para aqueles que não têm acesso a um exame ecocardiográfico, utilizarem o método VLAS para seguir a evolução do tamanho do átrio esquerdo durante a progressão da DDVM. Também se verificou que as medições VLAS têm uma correlação positiva com as medidas ecocardiográficas do átrio esquerdo, o que implica que quando uma medida aumenta a outra aumenta também, e vice-versa.

Palavras chave: Doenças Degenerativa da Válvula Mitral, Edema Pulmonar, cão, Vertebral Heart Score, Vertebral Left Atrium Size.

Abstract

Comparison of heart measurements in thoracic radiographs before and after the treatment of pulmonary edema in dogs with Degenerative Mitral Valve Disease: a retrospective study of 18 clinical cases

The Degenerative Mitral Valve Disease (DMVD) has the highest prevalence of all canine heart diseases accounting for 75-80% of the cases of dogs with cardiac disease.

DMVD is characterized by having an evolutive nature. As the disease progresses the microscopic and macroscopic alterations of the mitral valve's apparatus become more severe and gradually start preventing the valve's normal function. One of the complications that may occur is the development of pulmonary edema. Overt pulmonary edema occurs when the capacity of the pulmonary lymphatic system is exceeded, leading to an increase in the extravascular water content of the lungs.

The etiology and consequently the cure for DMVD are not currently known, hence the importance of understanding and developing tools that allow the monitoring of the disease. Even though the best way to assess and confirm the diagnosis of DMVD is through echocardiography, this exam requires additional expertise to be performed and interpreted, as well as substantial financial costs to the owner. Simultaneously, radiography of the thorax is widely available and cost-effective, which justifies the interest in studying the evolution of the radiographic measures Vertebral Heart Score (VHS) and Vertebral Left Atrium Size (VLAS) in dogs with DMVD.

This retrospective study aims to compare heart measurements in thoracic radiographs before and after the treatment of pulmonary edema in 18 dogs with DMVD that were submitted to consultation in a french veterinary referral center.

The main conclusion of this study is that the size of the left atrium and the cardiac silhouette decreases after the resolution of cardiogenic pulmonary edema when compared to the dimensions during its occurrence. Furthermore, this decrease in the left atrium's size is detectable using the VLAS method, which confirms its value in monitoring the progression of the disease. Consequently, it is possible for those who do not have access to an echocardiographic exam, to use the VLAS method to follow the evolution of the left atrium's size throughout the progression of DMVD. It was also verified that VLAS measurements have a positive correlation with echocardiographic measures of the left atrium, implying that when one increases the other does so as well, and vice-versa.

Key Words: Degenerative Mitral Valve Disease, Pulmonary Edema, dog, Vertebral Heart Score, Vertebral Left Atrium Size.

Résumé

Evolution de la cardiomégalie évaluée par radiographie thoracique chez des chiens atteints de Maladie Valvulaire Dégénérative Mitrale avant et après le traitement de l'œdème pulmonaire : une étude clinique rétrospective de 18 cas

La Maladie Valvulaire Dégénérative Mitrale (MVDM) est la cardiopathie la plus fréquent dans l'espèce canine, représentant 75-80% des cas.

La MVDM est caractérisée par sa nature évolutive. Avec la progression de la cardiopathie, les altérations microscopiques et macroscopiques de la valve mitrale deviennent plus importantes et commencent graduellement à empêcher le fonctionnement normal de la valve. La complication la plus fréquente de cette maladie dégénérative est le développement d'un œdème pulmonaire qui se produit lorsque la capacité du système lymphatique du poumon est dépassée, entraînant l'accumulation de contenu aqueux dans le compartiment extravasculaire.

L'étiologie et donc par conséquent le traitement curatif de la MVDM ne sont pas actuellement connus, d'où l'importance de comprendre et développer des outils permettant un suivi optimal de la maladie. Bien que l'examen de choix pour confirmer et grader le stade de la cardiopathie soit l'échocardiographie, cet examen représente un investissement certain pour le propriétaire, nécessite un matériel onéreux et demande une certaine expérience pour sa réalisation et interprétation. À l'inverse, l'imagerie thoracique par la réalisation de clichés radiographiques thoraciques est une technique largement disponible et moins coûteuse, justifiant son intérêt pour l'étude de l'évolution de certains indices comme le *Vertebral Heart Score* (VHS) et le *Vertebral Left Atrium Size* (VLAS) chez les chiens atteints de la MVDM.

L'objectif de cette étude rétrospective était de comparer ces deux indices sur les radiographies thoraciques avant et après le traitement de l'œdème pulmonaire chez 18 chiens atteints de MVDM présentés en consultation dans un centre hospitalier vétérinaire de référence français.

Cette étude a permis de démontrer que le diamètre de l'atrium gauche et de la silhouette cardiaque diminue après la résolution d'un œdème pulmonaire d'origine cardiogénique en comparaison avec les données recueillies au moment de cette insuffisance cardiaque congestive. De plus, cette diminution de l'atrium gauche est détectable par la méthode VLAS, ce qui confirme la valeur de cet indice pour la surveillance de la progression de la maladie. Enfin, cette étude a également démontré que les mesures VLAS sont corrélées positivement aux valeurs de diamètre atrial gauche obtenues par échocardiographie confirmant le pouvoir diagnostique de cet indice. Ces données ouvrent donc la perspective d'utiliser la méthode VLAS pour suivre l'évolution du diamètre atrial gauche au cours de la progression de la MVDM pour les personnes n'ayant pas accès à un examen échocardiographie

Mots clés : Maladie Valvulaire Dégénérative Mitrale, œdème pulmonaire, chien, Vertebral Heart Score, Vertebral Left Atrium Size.

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List of Abbreviations

% - Percentage
μg - Microgram
2-D - Two-dimensional
5-HT - Serotonin, 5-hydroxytryptamine
A - Age
ACEI - Angiotensin Converting Enzyme Inhibitor
ACVIM - American College of Veterinary Internal Medicine
ANP - A-type natriuretic peptide
Ao - Aortic Root
BNP - B-type natriuretic peptide
Bpm - Beats per minute
CF - Cardiac Frequency
CHF - Congestive Heart failure
ChuvA - Hôpital des Animaux de Compagnie du Centre Hospitalier Universitaire Vétérinaire d'Alfort
CHV-Frégis - Centre Hospitalier Vétérinaire Frégis
CKCS - Cavalier King Charles Spaniel
CRI - Continuous Rate Infusion
CVHD - Chronic Valvular Heart Disease
D - Day
D0 - Day 0, the day when the first set of thoracic radiographs was taken, during the occurrence of pulmonary edema
D1 - Day 1, the day when the second set of thoracic radiographs was taken, during the absence of pulmonary edema
DMVD - Degenerative Mitral Valve Disease
DV - Dorsoventral
DVHS - Difference between the VHS measurement between D1 and D0
DVLAS - Difference between the VLAS measurement between D1 and D0
ECG - Electrocardiogram
IM - Intramuscular
IV - Intravenous
Kg - kilogram
LA - Left Atrium
LA/Ao - Ratio between the diameter of the Left Atrium and the Aortic root
LHM - Left Heart Murmur

LV - Left Ventricle

Mg - Milligram

Min - Minute

ND - Number of days between the presence and the absence of pulmonary edema

Nº Obs. - Number of Observations

NT-proANP - N-terminal pro-A-type natriuretic peptide

NT-proBNP - N-terminal pro-B-type natriuretic peptide

NYHA - New York Heart Association Functional Classification

PISA - Proximal Isovelocity Surface Area

PO - *per os*

Std. Dev. - Standard Deviation

T - Dose of the treatment with furosemide (mg/kg/day)

VD - Ventrodorsal

VHS - Vertebral Heart Score

VIC - Valvular Interstitial Cells

VLAS - Vertebral Left Atrial Size

W - Weight

Description of the Activities Performed during the Traineeship

The author's traineeship took place at two different instituions: *Centre Hospitalier Vétérinaire Frégis* (CHV-Frégis) located in Arcueil-France and *Hôpital des Animaux de Compagnie du Centre Hospitalier Universitaire Vétérinaire d'Alfort* (ChuvA) located in Maison Alfort-France. Both of the externships were performed under the Erasmus mobility program.

The curricular externship was conducted at the CHV-Frégis and it was where the author engaged in the redaction of this dissertation under the supervision of Dr. Cecile Damoiseaux. It had a total duration of four months starting on the 1^{st} of October 2018 and ending on the 28^{th} of January 2019, totalizing approximately 756 hours. The first two months were spent in the service of Surgery and the last two at the service of Internal Medicine.

As far as the traineeship at ChuvA is concerned it was a two month-long extracurricular externship which began on the 4^{th} of February 2019 and ended on the 5^{th} of April 2019. A total of 385 hours were performed under the orientation of Dr. Céline Robert, based on weekly rotations. The services attended were Dermatology, Preventive Medicine, Reproduction, Surgery, Elective Surgery, Internal Medicine, Ophthalmology, Neurology, Cardiology, Exotic Animals, Physiotherapy and Rehabilitation. Overall, the externship activity of the author made up 6 months, counting a total of 1141 hours.

Activities performed at the Centre Hospitalier Vétérinaire Frégis

As previously mentioned, the author was mainly in two services, Surgery and Internal Medicine. In both of them the daily routine started at 7.30am in the respective hospitalization ward. A general examination was performed to each hospitalized patient by the author and the intern (a recently graduated clinician undergoing a multidisciplinary formation) appointed to the service. After all the exams were done, a medical round started at around 8.00am in the presence of the day-time and night-time intern, surgery/ internal medicine clinicians, residents, board-certified specialists, a nurse and the trainee. This was the moment when a detailed presentation of each animal was made by the clinician responsible for the case or by the night-time intern in the event that it was a patient who was hospitalized during the night shift. The attending specialist then proceeded to demand the elaboration of a differential diagnosis list and all the group participated in the discussion of the complementary exams, surgeries and treatments to perform during the day. Afterwards the tasks requested from the trainee differed according to the service.

In the Surgery service, once the round was over, both the trainee and the intern helped the responsible nurse with the application of bandages, management of fluid therapy and administration of treatments. It was also part of the activities to perform complementary exams such as the collection of blood samples, measurement of blood pressure, electrocardiograms, packed cell volume, biochemical and urinary rapid parameters. When at the surgical theater, the author helped

getting the animals ready for surgery, amongst her functions were: preparing the anesthesia and pre-medication, administering the drugs, intubating the patient, doing the asepsis of the surgical field and transferring the animal onto the surgical table. The author was also able to participate assistant surgeon in multiple soft tissue and orthopedic surgeries in alternation with the intern. As examples of surgeries assisted by the author are: laparoscopic ovariectomies, caesarian sections (and respective neonatal reanimation), correction of brachycephalic syndromes with a CO_2 laser, removal of intestinal foreign bodies, removal of cranial meningiomas, correction of patent *ductus arteriosus*, thoracotomies, cystotomies, ligation of portosystemic shunts, correction of ectopic ureters, diaphragmatic and perineal hernias, Tibial Plateau Leveling Osteotomies, corpectomies, hip replacements, fracture assessment and corrections, amongst others. During the surgeries in which the author did not participate she could, nonetheless, observe and take the opportunity to discuss with the surgeons the surgical techniques and other aspects of the pre- and post-operative care. Concerning this last aspect, it was also the intern and the trainee's responsibility to ensure the short-term post-operative care, which gave the author a more profound understanding of both post-operative and pain management. If there were spare time between surgeries the trainee was given the opportunity to attend consultations, which were highly beneficial in understanding the entire path that leads to a surgery and to further discuss the cases with the specialists. Moreover, to the author's opinion, the chance to learn how to best approach the patients' owners was very enriching.

In the service of Internal Medicine, after the round, the trainee alongside the intern carried out the complementary exams previously discussed with the medical team. Some examples of the activities undertaken were: collection of blood samples, measurement of blood pressure, packed cell volume, biochemical and urinary rapid parameters, insertion of vascular and urinary catheters, nasoesophageal feeding tubes, collection of urine through cystocentesis, echographic assessment of the Left Atrium/ Aorta ratio and blood type typification. Furthermore, the author helped with the positioning of patients for ultrasonographical examination, both abdominal and cardiac, management of fluid therapy, drug administration and blood transfusions. In the event of an animal entering in cardiorespiratory arrest, the author helped not only with the reanimation maneuvers but also with the preparation of the drugs needed. Once the management of all the hospitalized animals were assured, as in the Surgery service, the trainee could go and attend consultations. This represented an opportunity to debate multiple arrays of clinical cases with highly graduated clinicians, previously and after the consultations, which enabled the acquirement of a rigorous diagnostical approach and management of a multitude of situations. Since the author has a special interest in Cardiology, she spent some of this free time attending the service's consultations and echocardiographies. Furthermore, during the afternoon the trainee had the chance to occasionally assist gastrointestinal endoscopies, transtracheal washes and bronchoscopies, rhinoscopies, biopsies, cytologies, enemas, cerebrospinal fluid collections and thoracentesis.

Lastly, in both services, a second medical round was performed at around 20.00pm where the general status and results of the complementary exams of all hospitalized animals were discussed. In addition, some considerations were given by the specialist concerning the prognosis and future procedures to undertake.

Activities performed at the Centre Hospitalier Universitaire Vétérinaire d'Alfort

In ChuvA the working dynamics were different from CHV-Frégis. There were weekly rotations in the various services in which the author was inserted in a group of 3-4 students. The tasks required from the students started by individually receiving the patients and doing a pre-consultation that consisted in taking the medical history, performing a general clinical examination and basic laboratory testing. Afterwards, the author presented the case to a clinician formulating a diagnose, proposing a justified treatment plan and recommending preventive measures. Furthermore, when complementary exams were needed it was also the student responsible for the case who did them. At the end of each consultation, the author wrote the respective clinical report. The students had the liberty to discuss the clinical case with each other and the clinicians and, some topics were further debated in almost daily small lectures with the group. The routine described is applicable to all the services apart from Elective Surgery, Physiotherapy and Rehabilitation.

The first week was carried out in the Dermatology department where the author collected many skin and fur samples which were then stained by Gram technic and analyzed. It was a great opportunity to deepen and consolidate the author’s knowledge in this field as well as being in touch with multiple common and rare cases.

The second week took place at the service of Preventive Medicine. It was there that the author learned about some of the country’s legislation and vaccination plans. She had the responsibility of choosing the plan accordingly to the animal’s needs, introducing the electronic identification, fill in passports and prescribing the accurate anti-parasitic treatment.

At the service of Reproduction, the author had the opportunity to assist to artificial inseminations, collecting of semen samples, gestational echographies and monitoring of reproductive cycles. In the author’s view, the time spent at this service was extremely enriching since she was in touch with board-certified specialists and residents that frequently took the time to explain in detail the procedures and further instruct the students in plenty of reproduction related subjects.

During the time spent at the service of General Medicine, the author was faced with a multitude of cases, which were amply discussed with the clinicians. Every time an animal needed hospitalization, radiographic or echographic exams, it was the student’s responsibility to accompany the animal during all its time in the hospital. This was highly educative since it let the students follow a case and acquire a sense of responsibility towards the animal they receive in pre-consultation.

In the week conducted in the service of Medicine of Specialties the author passed through the departments of Cardiology, Ophthalmology, Exotic Animals and Neurology. In Cardiology the

students visualized the realization of various echocardiographic exams and electrocardiograms. They were also expected to analyze treatment plans and suggest eventual adaptations based on the patients' needs. In Ophthalmology the author was able to perform exams such fundoscopic exam, Schirmer's test, fluorescein test, testing of reflexes, amongst others, providing an excellent environment to better her skills in doing a complete ophthalmologic exam. Regarding the Exotic Animals department, the author performed pre-consultations both in small mammals and birds, performed examinations of rabbit's teeth, and learned the vaccination plans of all the animals most commonly presented in consultation. In Neurology, the presentation of the cases was done in a small amphitheater. It was requested of the students to elaborate a timeline reporting all the epileptic episodes of the animals as well as explaining all the treatment modifications. In this service the author learned how to properly perform a detailed neurologic exam and interpret its results.

As far as the time spent in the service of Surgery is concerned, the author did the consultations pre-surgery, mainly orthopedic. Consequently, she improved her skills in performing a complete orthopedic exam and enhanced her knowledge in surgical techniques, having the opportunity to discuss which was the best approach to each presented problem. In the context of this area, the students attended sessions of physiotherapy and rehabilitation such as hydrotherapy, electric stimulation and massages.

When in the Elective Surgery service, the author had the chance to perform cat's sterilizations and castrations in first hand. She had responsibility over the animal from the moment she talked to the patient's owners and executed a general examination pre-surgery, during the anesthetic induction, the surgical act and the post-operative care. Furthermore, she assisted and was second hand to other surgeries such as removal of mammary chains, abdominal testicles and pyometras.

Last but not least, the author spent an entire week in the service of Cardiology. There she was given the chance to learn how to manipulate an ultrasonographic transducer and correctly conduct an echocardiographic exam. She was also confronted with the interpretation of multiple electrocardiograms and was given several small lectures related to the cases presented in consultation. Moreover, the author had the opportunity to participate in a professional formation of level two echocardiography lectured by Dr. Valerie Chetboul, a board-certified cardiology specialist. This formation had a theoretical part, where the different echocardiographic views and the Doppler technique were copiously reviewed, and a second part where the author had the chance to put into practice what was previously debated.

1. Introduction to the Dissertation

The present Veterinary Medicine Master's Dissertation represents the culmination of a continuous learning process started in 2013 in the Faculty of Veterinary Medicine, Lisbon's University. The theme and elaboration of this dissertation are the outcome of knowledge acquired during the curricular externship at CHV-Frégis. Even though it was a multidisciplinary traineeship, the manifested interest of the author in the discipline of Cardiology made her choose a supervisor in this area. Ergo, both the dissertation and the curricular externship were done under the supervision of Dr. Cecile Damoiseux, board-certified specialist in Cardiology. The choosing of the theme was a combined effort between her and the author.

Given the fact that Degenerative Mitral Valve Disease (DMVD) has the highest prevalence of all canine heart diseases, but a medical cure is not available in the present days, it is of extreme importance to understand what tools allow the monitoring of the disease (Das & Tashjian, 1965; Egenvall, Bonnett, & Häggström, 2006; Gordon, Saunders, & Wesselowski, 2017). Consequently, this theme was selected with the purpose of further enriching the knowledge about the variation of heart measurements in thoracic radiographs over the period of progression of DMVD, specifically before and after the treatment of pulmonary edema.

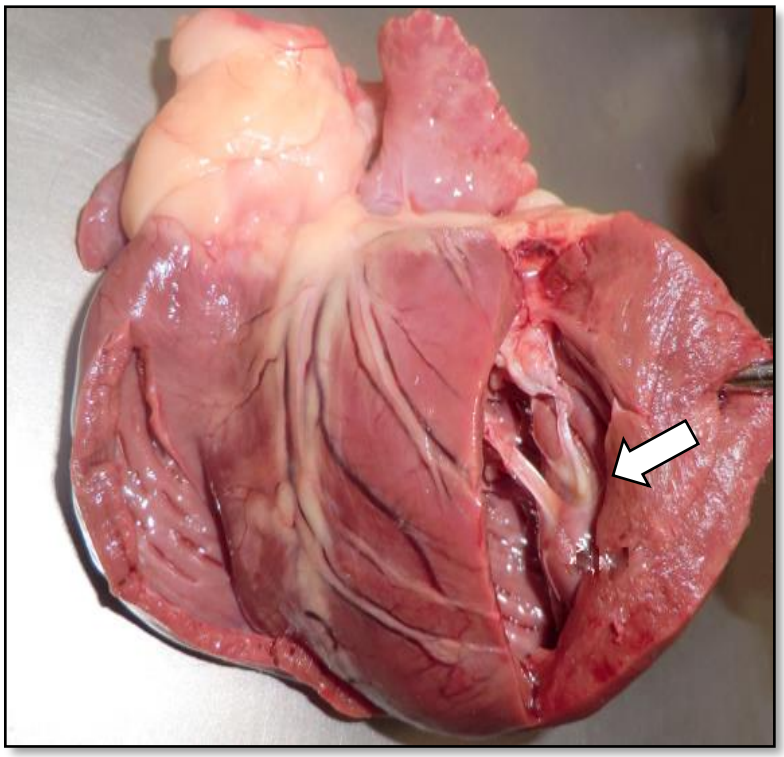
2. Degenerative Mitral Valve Disease

2.1 Introduction

The heart is a specialized muscular organ that rhythmically contracts and pumps blood from the low-pressure venous side to the high-pressure arterial side of the circulation. Efficient pumping occurs because of the orderly contraction sequence of the different heart chambers and the presence of valves inside the heart that ensure an unidirectional blood flow (Klabunde, 2012).

There are four heart chambers: right atrium, right ventricle, left atrium (LA) and left ventricle (LV). The right atrium receives the blood from the anterior and posterior *vena cavae* returning from the systemic circulation. Blood flows from the right atrium, across the tricuspid valve (right atrioventricular valve), and into the right ventricle. The outflow vessel of the right ventricle is the pulmonary artery, which is separated from the ventricle by the semilunar pulmonary valve. Blood returns to the heart from the lungs via four pulmonary veins that enter the left atrium. Blood flows from the LA, across the mitral valve (left atrioventricular valve), and into the LV, it is then ejected into the aorta across the aortic valve (Klabunde, 2012). A healthy dog's heart is shown in figure 1.

Figure 1: Healthy dog's heart from a Boxer with both ventricles sectioned, left-side view. Original image, kindly provided by Doctor Graça Pires, professor of Anatomy in the Faculty of Veterinary Medicine, Lisbon's University.



Legend: The white arrow indicates the point of insertion of the *chorda tendinea* in the papillary muscles, both of them components of the mitral valve apparatus.

For the purpose of maintaining a working circulatory system, the heart needs to ensure two fundamental mechanical functions. One is to fulfill the perfusion requirements of the metabolizing tissues by ejecting a sufficient amount of blood at the right pression into the aorta and pulmonary arteries. The other is to provide drainage of the capillary beds preserving an appropriate distribution of the circulating blood pool (Ljungvall & Häggström, 2016).

Approximately 10% of the dogs presented to primary care veterinary practices have heart disease, being Chronic Valvular Heart Disease (CVHD) the most common one (Atkins, et al., 2009). In CVHD, the left atrioventricular or mitral valve is more often affected and to a greater degree, although in approximately 30% of cases the right atrioventricular (tricuspid) valve is also involved (Borgarelli & Buchanan, 2012; Garncarz, Parzeniecka-Jaworska, Jank, & Łój, 2013). However, isolated degenerative disease of the tricuspid valve is uncommon. Thickening of the aortic and pulmonary valves is sometimes observed in older animals but rarely causes more than mild insufficiency (Ware, 2014).

DMVD of the left atrioventricular valve is characterized by the accumulation of glycosaminoglycans (myxomatous proliferation) and fibrosis of the valve leaflets and *chordae tendineae* (Häggström, 2010). The condition has been given numerous designations on the basis of its clinical and pathologic features. The terms chronic degenerative valvular disease, chronic valvular disease,

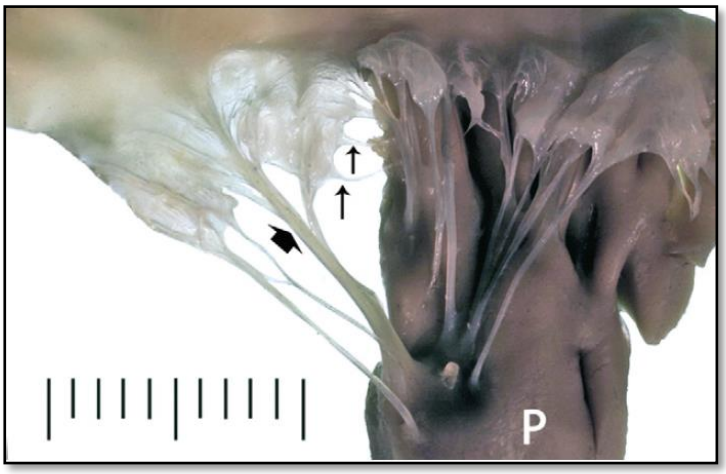
chronic valvular fibrosis, endocardiosis, myxomatous mitral valve disease, myxomatous valvular degeneration, myxomatous transformation, and mucoid degeneration all refer to the same disorder (Häggström, 2010; Abbott, 2016; Ljungvall & Häggström, 2016).

2.2 Mitral valve's anatomy and histology

2.2.1 Anatomy

The mitral valve's competence depends on the functional and structural performance of six basic components that form the mitral valve apparatus: 1) the posterior left atrial wall, 2) the mitral valve annulus, 3) the mitral valve leaflets, 4) the *chordae tendineae*, 5) the left ventricular papillary muscles, 6) and the associated left ventricular wall (see figure 2). Each of these elements act both independently and synergistically, working in a complex interplay with the objective of preserving the valve's integrity (Perloff & Roberts, 1972; Fox, 2012).

Figure 2: Dorsolateral view of a normal mitral valve apparatus from two-year-old mongrel dog (Reproduced with permission from Dr. Philip Fox)



Legend: Along the top frame are the valve leaflets attached to the myocardial tissue. Primary *chordae tendineae* (small vertical arrows) and secondary *chordae tendineae* (broad arrow) attach the mitral leaflets to the papillary muscle (P), Scale, mm (Fox, 2012).

There are two mitral valve leaflets, the anterior (septal) and the posterior (parietal), being the juncture where they are supported at their hinge points referred to as the mitral annulus. This discontinuous fibrous ring is a dynamic structure that suffers changes in shape and size during the cardiac cycle (Glasson, et al., 1996; Fox, 2012). In healthy animals, the mitral leaflets are thin, translucent structures, with a rough zone near their free margin where *chordae tendineae* attach, and a smooth zone towards the annular junction (Shimakura, Ishihara, Kawazoe, & Hashimoto, 1978; Fox, 2012; Abbott, 2016).

Intact mitral valve *chordae tendineae* mediate efficient and forceful ventricular contraction and optimize left ventricular systolic performance. Each mitral valve leaflet is adjoined to the anterior and posterior papillary muscles or occasionally, directly to the ventricular wall by these fibrous chords.

Together, these structures work in a coordinated manner to prevent mitral valve prolapse and regurgitation (Oe, et al., 1993; Fox, 2012).

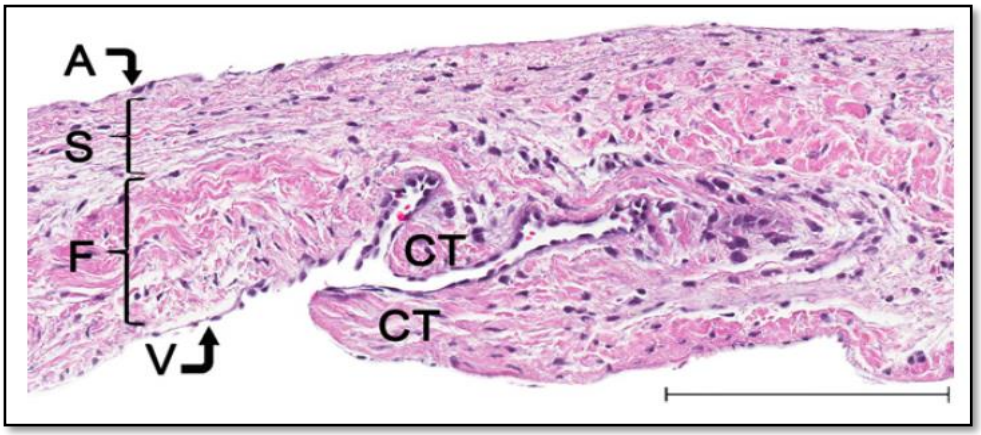
Chordae tendineae are categorized according to their insertion sites on the mitral valve's leaflets, as a result, there are two major sets of *chordae*. Primary (first-order or marginal) *chordae* arise from the papillary muscles and attach to the free margins of the leaflets, they are thin and most numerous. Secondary (second-order) *chordae* also ascend from the papillary muscles but insert near the intersection of the rough and smooth zone, on the ventricular aspect of the valve leaflets; they are larger and fewer in number. Both types of *chordae*, however, may originate from a common bifurcating stem (Rodriguez, et al., 2004; Fox, 2012).

Primary and secondary *chordae* serve different roles (Ibadia, Casali, Chassignolle, & Janier, 1997). The primary *chordae* maintain leaflet apposition and facilitate valve closure, whereas secondary *chordae* are believed to be involved in maintaining normal LV size and geometry. When a primary *chorda* gets ruptured, it results in acute mitral regurgitation. Sectioning secondary *chordae*, in contrast, does not produce mitral regurgitation, but can impact ventricular geometry and systolic function. (Nielsen, et al., 2001; Silbiger & Bazaz, 2009).

2.2.2 Histology

The mitral valve's leaflets are heterogenous and laminated structures in which four distinct layers can be differentiated: atrialis, spongiosa, fibrosa, and ventricularis (layers from the atrial to the ventricular aspect) (see figure 3). These are most prominent at the leaflets mid-portion (Aupperle, et al., 2009).

Figure 3: Photomicrograph of the proximal third of the posterior mitral valve leaflet from a three-year-old German Shepherd dog. (Reproduced with permission from Dr. Philip Fox)



Legend: Layers of the mitral valve leaflets: atrialis (A), spongiosa (S), fibrosa (F) and ventricularis (V). CT stands for *chorda tendinea*. Haematoxylin and eosin stain. Bar = 200µm (Fox, 2012).

The atrialis consists in a thin layer of endothelial cells supported by scattered collagen fibers, elastic fibers, fibroblasts and smooth muscle cells. The spongiosa extends from the annulus to the free edge of the leaflet, and it is characterized by being a layer rich in proteoglycans and glycosaminoglycans, it also contains ground substance embedding a loose collection of collagen,

elastic fibers, fibroblasts, and Anichkov's cells. The fibrosa is composed by a dense layer of compact collagen bundles with scattered fibroblasts that proximally has continuity with the annulus, while distally continues with the central core of *chordae tendineae*. The ventricularis, is a thin layer alike the atrialis but without smooth muscle cells (Aupperle, et al., 2009). The endothelial covering of the ventricular aspect of the leaflets and the one covering the *chordae tendineae* are continuous, and the fibrous valvular layer is continuous with the fibrous cardiac skeleton (Fox, 2012). Amongst the roles of the fibrous skeleton are to internally reinforce the myocardium, to anchor the valve cusps, to avoid excessive dilation of valvular orifices, to serve as an insertion point for atrial and ventricular myocyte bundles, and to buffer conduction of electrical impulses between atria and ventricles (Evans & Lahunta, 2013).

2.3 Epidemiology

DMVD has the highest prevalence (percentage of a population with a particular disease at a given point in time) of all canine heart diseases, accounting for 75-80% of the cases of dogs with cardiac disease (Das & Tashjian, 1965; Egenvall, et al., 2006; Ljungvall & Häggström, 2016). Due to the fact that DMVD is a progressive disease, a clinically evident valvular dysfunction is preceded by subtle changes in the valve structure. As a result, the prevalence of DMVD reported by clinical studies is lower than that detected by postmortem examination (Abbott, 2016).

The disease is age related and the prevalence increases drastically in 4 to 5-year old dogs (Whitney, 1974). Concerning the sex, DMVD is approximately 1,5 times more common in males than in females, furthermore the disease seems to appear at a younger age and have a more rapid progression from mild to severe in males. Given the fact that the disease affects middle-aged to old dogs, the animal can succumb to other co-morbid conditions or old age before progressing into congestive heart failure (Thrusfield, Aitken, & Darke, 1985; Häggström, Hoglund, & Borgarelli, 2009; Borgarelli & Häggström; 2010).

DMVD can be encountered in all breeds, but the highest prevalence is found in small to medium-sized dog breeds, namely Cavalier King Charles Spaniels (CKCS), Dachshunds, Chihuahuas, Miniature Poodles, Miniature Pinchers, Beagles, Whippets and Cocker Spaniels. The coat color does not seem to have an influence in the frequency of the valve disease (Borgarelli & Buchanan, 2012). Even though most studies focus in smaller breeds, when larger breeds are taken into account German Shepherd dogs appear to be over-represented in the experimental groups. Additionally, larger dogs often experience faster disease progression allied with a greater myocardial dysfunction, and a more guarded prognosis (Madron, 1992; Borgarelli, et al., 2004). In small breed dogs, the progression is usually slow but can be, at times, unpredictable. CKCS are remarkably predisposed to developing DMVD at a younger age, nonetheless the time course of their disease progression to heart failure does not appear to be noticeably different from that of other small breed dogs (Keene, et al., 2019).

2.4 Etiology

First of all, it is important to keep in mind that a definitive etiology to the DMVD is, to this date, unknown. Despite that fact, several possible causes have been suggested throughout the years (Ljungvall & Häggström, 2016).

Since some dog breeds are more predisposed to an early onset of DMVD, it is believed that the disease has a strong genetic background, being heritable in at least some breeds, namely the CKCS and the Dachshund (Olsen, Fredholm, & Pedersen, 1999; Lewis, Swift, Woolliams, & Blott, 2011; Meursa, et al., 2018). DMVD has been suggested to be inherited as a polygenic threshold trait, which means that multiple genes influence the trait and a certain threshold has to be reached before the disease develops (Swenson, Häggström, Kvart, & Juneja, 1996; Olsen, et al., 1999). Moreover, an association between the presence and severity of DMVD in parents and offspring at a given age, has been demonstrated. In other words, when both parents have an early onset of the disease, the offspring will, on average, develop the disease at a younger age as well. On the contrary, when both parents have a late onset of DMVD, they will give birth to offspring that manifests the disease at an old age or never (Lewis, et al., 2011). With regard to the threshold, males have a seemingly lower one than females, as a consequence they are expected to manifest the disease at a younger age than females, within a family in which the offspring have approximately the same genotype (Ljungvall & Häggström, 2016). A genome-wide analyses of CKCS unveiled 2 loci associated with the development of DMVD, however, even though the chromosomal regions have been identified, no specific causative genes have been determined (Madsen, et al., 2011). These discoveries reinforce the hypothesis that a genetically determined process initiates the valve degeneration (Swenson, et al., 1996; Olsen, et al., 1999; Madsen, et al., 2011).

Another suggested cause for DMVD is related to phenotypic alterations in the valvular interstitial cell (VIC) population. A transformation seems to occur from an inactive fibroblast phenotype, which is responsible for normal valve structure by maintaining the extracellular valve matrix, to a more active myofibroblast type. This happens both in dogs with DMVD and in humans with the equivalent acquired valvular disease (Rabkin, et al., 2001; Black, French, Dukes-McEwan, & Corcoran, 2005). As it will be explained below in greater detail, the degenerative changes of DMVD are characterized by an overproduction and deposition of extracellular matrix with disruption of collagen content and organization in the mitral leaflets and *chordae tendineae* (Han, et al., 2008; Aupperle, et al., 2009). This remodeling, that happens within the mitral valve, is mediated by the referred activation of the normally quiescent VIC (Oyama & Chittur, 2006; Disatian, Ehrhart, Zimmerman, & Orton, 2008). Serotonin (5-hydroxytryptamine, 5-HT) has been linked to VIC activation in several species, including humans, rats, and dogs. Additionally, multiple studies have shown altered local 5HT signaling in dogs with DMVD (Oyama & Chittur, 2006; Connolly, et al., 2009; Disatian & Orton, 2009; Oyama & Levy, 2010; Scruggs, Disatian, & Orton, 2010; Orton, Lacerda, & MacLea, 2012).

Serotonin is a monoamine neurotransmitter known to control a wide range of biological functions (Jonnakuty & Gragnol, 2008). Most of 5-HT is synthesized in the intestine by the enterochromaffin cells, but there is also local synthesis of 5-HT in the heart (Ni & Watts, 2006; Lacerda, Maclea, Kisiday, & Orton, 2012). The 5-HT is then secreted into the blood stream, and nearly all of it is taken up by platelets via active transport and stored in its dense granules. Hence, serum concentration of serotonin results from an equilibrium between 5-HT synthesis, platelet uptake and storage, and metabolism (Launay, Schneider, Loric, Da Prada, & Kellermann, 2006). It has been shown that giving 5-HT injections to rats causes the development of valvular lesions (Gustafsson, et al., 2005). Moreover, valvulopathies in people have been associated with 5-HT-producing carcinoid tumors and the intake of serotonergic drugs (Lundin, Norheim, Landelius, Oberg, & Theodorsson-Norheim, 1988; Hendrikx, Van Dorpe, Flameng, & Daenen, 1996; Zanettini, et al., 2007; Gustafsson, Hauso, Drozdov, Kidd, & Modlin, 2008). There are many similarities between these 5-HT-induced valvular lesions and those in patients with DMVD, which further points towards serotonin having a role in the development of the disease (Disatian & Orton, 2009).

Furthermore, platelet 5-HT content is substantially higher in dogs with DMVD versus healthy non-CKCS dogs. When comparing healthy CKCS with healthy dogs of other breeds, it has been concluded that CKCS have a higher platelet 5-HT content, suggesting not only that platelet-derived 5HT is involved in the development of early stages of DMVD, but also that 5-HT may be genetically linked to the etiologic mechanisms of the disease in this breed. As for the 5-HT concentration in the mitral valve leaflets and left ventricle myocardium, it is also increased in affected dogs, denoting that altered tissue serotonin signaling is a specific feature of DMVD (Cremer, et al., 2014). However, serum 5-HT concentrations decrease with the increasing severity of the disease, which implies that even if serotonin primarily plays a role in the progression of early DMVD, it may be less involved when severe valvular lesions are already installed (Ljungvall, et al., 2013).

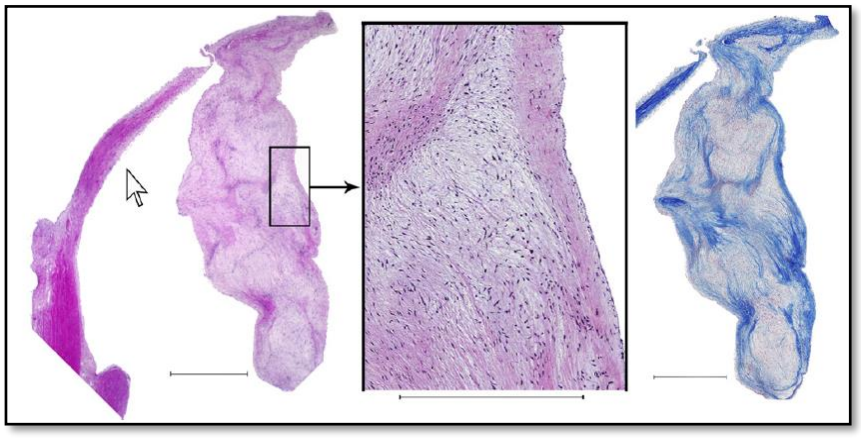
2.5 Natural History

2.5.1 Microscopic Alterations

As far as histopathological findings are concerned, a myxomatous degeneration starts to take place in the valve leaflets. This degeneration is characterized by giving origin to a disorganized and weakened connective tissue. There is a progressive expansion of the spongiosa layer that becomes thickened with increased extracellular matrix and proliferation of edematous ground substance. It is also verified that the normal arrangement of layered collagen in the fibrosa layer becomes disrupted and attenuated. In advanced stages, differentiating spongiosa and fibrosa layers can be challenging (see figure 4). Increased amounts of mucopolysaccharides, and glycosaminoglycans are frequently seen within affected valves (Buchanan, 1977; Han, et al., 2008; Hadian, Corcoran, & Bradshaw, 2010; Han, Black, Culshaw, French, & Corcoran, 2010). Regarding the heart chambers, changes in the composition and structure of the extracellular matrix and the myocytes are also present. In

advanced stages of DMVD the presence of myocardial fibrosis and intramyocardial arteriosclerosis have been reported, particularly in the papillary muscles (Falk, Jönsson, Olsen, & Pedersen, 2006).

Figure 4: Photomicrograph of the distal posterior mitral valve leaflet from a twelve-year-old Maltese dog with severe Degenerative Mitral Valve Disease (DMVD) (Reproduced with permission from Dr. Philip Fox)



Legend: It can be observed the increased thickness of the spongiosa from glycosaminoglycan and proteoglycan deposition, and degeneration of the fibrosa. Left frame: Haematoxylin and eosin stain; bar = 1mm. Right frame: Masson trichrome stain; bar = 1 mm. This image demonstrates the total loss of the leaflet's normal layered arrangement in DMVD. The white arrow points to a secondary *chorda tendinea* (Fox, 2012).

2.5.2 Macroscopic Alterations

DMVD is characterized by having a long pre-clinical asymptomatic period, in which the first morphologic changes are only visible on the necropsy (Borgarelli & Buchanan, 2012). Due to the evolving nature of the DMVD, with the advancing of time the lesions become more observable and, depending on the stage of the disease, the valve will present different macroscopic and microscopic appearances (Ljungvall & Häggström, 2016).

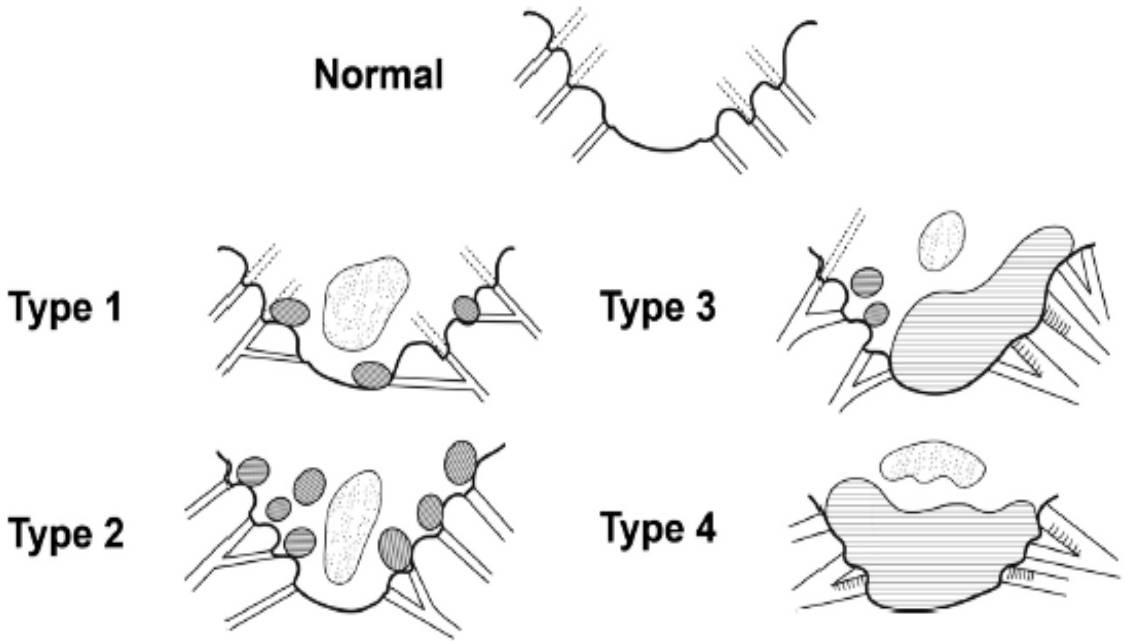
The first macroscopic modifications occur in the area of apposition of the leaflets, being generally more evident in the sites where *chordae tendinea*e insert, nonetheless the *chordae* themselves can also be affected by the myxomatous degeneration. The normally thin and translucent free edges of the leaflets gradually become thickened and irregular with parts bulging towards the LA side (Han, et al., 2013; Corcoran, et al., 2004). Progressively the lesions spread to other zones of the leaflets and the bulging worsens. Succinctly, DMVD is macroscopically characterized by nodular distortion and thickening of the mitral leaflets and *chordae tendinea*e, beginning with a few nodules at the free edges of the valve that later increase in number and coalesce to form plaque-like elevations (Whitney, 1974; Buchanan, 1977).

In 1974, Whitney tabulated the necropsy findings of several dogs with DMVD and elaborated a system to classify the different stages of degenerative mitral lesions, that demonstrates the evolving nature of the lesions. This system devises the lesions in four types (see figure 5). Type I lesions are those in which a few small edematous nodules can be found in the area of apposition of the leaflets,

at this point the *chordae tendineae* themselves are not affected and the valve maintains its competence. In Type II lesions the nodules are bigger but the *chordae tendineae* remain unaffected. As for Type III lesions, the nodules are larger and plaque-like elevations can be seen, moreover the *chordae tendineae* are thickened and irregular proximally, being at this stage that evidence of valvular incompetence starts to show. Finally, in Type IV lesions the valve is severely distorted and contracted, the *chordae tendineae* are affected in all surface and may even be ruptured, in addition there is evidence of valvular incompetence in the majority of the cases (Whitney, 1974).

In patients presenting severe cases of DMVD, the atrial wall may rupture to different extents causing hemopericardium and acute left heart failure. Endomyocardial splits, ruptured pectinate muscles in the atrial appendage and acquired atrial septal defects may be observed in such cases (Buchanan, 1972). In case of rupture of the atrial septum, although uncommon in dogs with DMVD, the acquired septal defect normally leads to right failure instead of acute left heart failure (Peddle & Buchanan, 2010).

Figure 5: Four grade classification of mitral valve lesions according to Whitney. (Reproduced with permission from Dr. Michele Borgarelli)



Legend: The shaded areas represent nodular or diffuse thickening of the valve. The dotted areas represent zones of opacity caused by myxomatous degeneration (Borgarelli & Buchanan, 2012).

2.5.3 Physiopathology

All of the mentioned alterations in patients with DMVD generate a distortion of the valve architecture and *chordae tendineae*, which leads to a defective coaptation of the mitral leaflets during ventricular systole and a possible systolic atrial displacement of the valve leaflets. In healthy individuals, during early systole, the mitral leaflets are forced into apposition when the left ventricle pressure surpasses the left atrial pressure avoiding the retrograde ejection of blood. Additionally, the tethering effect of

the *chordae tendineae* averts the prolapse of the leaflets into the LA. In dogs with DMVD, due to the abnormal coaptation, not all of the left ventricular stroke volume is ejected through the aorta, a percentage flows backwards into the LA. Moreover, the elongation or rupture of the *chordae tendineae* causes the leaflets to completely or partially prolapse, further aggravating the mitral regurgitation (Beardow & Buchanan, 1993; Abbott, 2016). The importance of the regurgitation volume depends not only on the size of the regurgitant orifice, but also on the systolic pressure gradient between the left heart chambers (Buchanan, 1977; Ahmed, McGiffin, O'Rourke, & Dell'Italia, 2009).

The consequences of mitral regurgitation vary according to its severity. A mild regurgitation is not sufficient to induce changes in the heart size and function, given the fact that the small volume that goes backwards is effortlessly accepted by the LA and the volume going in the right direction is preserved. Meanwhile, with the progression of the valve's lesions, the regurgitant volume becomes greater, triggering cardiac and non-cardiac compensatory mechanisms whose objective is to maintain the forward stroke volume (Baumgartner, 2017). In dogs with advanced stages of DMVD, more than 75% of the total LV stroke volume can be ejected back into the LA during systole (Kittleson & Brown, 2003).

Once the mitral valve starts leaking, the blood entering the LV during diastole comes not only from the lungs but also from the fraction that has been previously regurgitated into the LA, which causes an increase in the preload and a reduction of the after load (resistance to LV emptying) (O'Gara, et al., 2008). In order to keep an intra-atrial pressure lower than that of the ventricle, the LA starts to enlarge in the interest of accommodating the increasing regurgitant volume and ensuring the right direction of the blood flow. Since the extent of the LA's enlargement is linked to the severity of the mitral regurgitation, it has been suggested that a major determinant factor to the degree of left-sided cardiac dilatation is the mitral regurgitation volume (Kittleson & Brown, 2003; Eriksson, Hansson, Häggström, Järvinen, & Lord, 2010). On the other hand, this capacity of enlargement relies on the compliance of the LA's walls, which depends on the regurgitant volume increase rate, that itself is determined by the rate of progression of the DMVD. In practice, this means that a dog with slowly progressing DMVD can present an extremely enlarged atrium without pulmonary congestion or edema. Whereas in cases with acutely increased mitral regurgitation volume, the LA does not have time to properly adapt its size. An abrupt raising of the pressure inside the LA that reflects backwards in the hydrostatic pressure of the pulmonary capillaries and is responsible for the development of pulmonary congestion and edema, which is defined as an increase in the extravascular water content of the lungs. Rupture of principal *chordae tendineae* is an example of a situation that can lead to this succession of events. Hence, it is clear that he LA's capacity to absorb the regurgitant volume is very important in protecting the pulmonary vascular bed from hypertension. Another compensatory mechanism that tries to delay the formation of a pulmonary edema is the development of a more effective lymphatic drainage of the pulmonary interstitium in chronic pulmonary congestion, since

overt pulmonary edema develops when the capacity of the pulmonary lymphatic system is exceeded (Staub, 1980; Murray, 2011; Borgarelli, et al., 2015 ; Ljungvall & Häggström, 2016).

As the degeneration of the valve advances, a progressively larger amount of blood moves ineffectually back and forth between the LV and the LA, diminishing the forward flow to the aorta. While the LA enlarges to accommodate the regurgitant volume, the LV has to compensate for the loss of forward stroke volume and deal with the high end-diastolic pressures and volumes. To do so, in response to the volume overload, the ventricle wall suffers an eccentric hypertrophy in which the ratio wall thickness and chamber size remains roughly unchanged. This hypertrophy maintains an adequate forward stroke volume and, at the same time, normalizes the pressure in the volume-overloaded LV (Carabello, 2002; Grossman & Paulus, 2013). The mechanism behind this is the Frank-Starling law, which dictates that when there is a greater end-diastolic volume the myocardium will be more stretched and, as a consequence, there will be an increased sarcomere length which enhances the sensitivity to calcium ions culminating in a much stronger contraction. In other words, the volume overloading causes an increase in the contractile strength and will increase the stroke volume (Komamura, et al., 1993). Hemodynamic wall stress has also been suggested to stimulate neurohormonal activation such as augmentation of the local production of angiotensin II that contributes to the myocardial hypertrophy. This production of angiotensin II is due to activation of the renin-angiotensin-aldosterone system by the increased secretion of renin in response to a decreased renal perfusion (Häggström, et al., 1997; Spinale, 2002).

All these cardiac and non-cardiac compensatory mechanisms try to provide the hemodynamic support needed to uphold the cardiac output in spite of the mitral regurgitation. Although beneficial at first, with the progression of the disease, they become contributing factors to the deterioration of the failing heart (Baumgartner, 2017). Concerning the cardiac remodeling, it is known that it will eventually impact the heart's mechanical function. For instance, the change in the dimensions of the cardiac chambers leads to an alteration in the overall shape of the heart from elliptical to globular. Consequently, even if it allows a myocardial adaptation to the anomalous wall stress, the disruption of the normal geometry of the mitral annulus may contribute to further aggravate the mitral regurgitation (Grossman & Paulus, 2013). Additionally, chronic volume overloading reduces cardiac function and myocyte contractility. This can be explained by a subendocardial ischemia caused by an increase in oxygen demand due to increased intramyocardial tension, neurohormonal activation and the Frank-Starling mechanism. Another contributing factor to the subendocardial ischemia is the enlarged muscle mass outgrowing the vascular growth. During ischemia there is a production of oxygen free radicals as well as nitric oxide activation which further aggravates the myocardial injury (Jennings & Reimer, 1981; Prasad, et al., 1996). The deterioration of systolic myocardial function in these conditions is a state sometimes referred to as overload cardiomyopathy (Abbott, 2016).

Nonetheless, the ventricular pump function is normally kept fairly well until late stages of DMVD, even in face of severe congestive signs (Ware, 2014). This explains why dogs with severe mitral

regurgitation have more commonly respiratory signs, caused by pulmonary congestion and edema, than signs caused by reduced forward cardiac output (Ljungvall & Häggström, 2016). As a consequence, death due to DMVD is most often mediated by congestive heart failure, even though sudden death can usually occur (Borgarelli, et al., 2008). Besides, overt heart failure tends to occur years after the start of the remodeling and myocardial disease, however, as demonstrated by a study, most cardiac enlargement takes place in the year preceding the onset of congestive heart failure (Lord, Hansson, Kvart, & Häggström, 2010).

2.5.4 Prognosis

Given the fact that the assessment of DMVD severity is not standardized, reports on the natural history and prognosis are rather variable (Keene, et al., 2019).

In a study including patients with several stages of DMVD, more than 70% of asymptomatic dogs with echocardiographic confirmation of the disease were alive at the end of 6.6 years of observation. The same study revealed that the survival time for those with moderate heart failure due to DMVD had a median survival time of 33 months and those with severe heart failure 9 months (Borgarelli, et al., 2008). Another study concluded that patients in pre-clinical stages had a survival time of 27.6 months and only 13% of dogs progressed to a more advanced stage during a 6.6 years period of time. Data coming also from this study demonstrated that over that period of observation the overall mortality was 27%, with cardiac deaths accounting for 11% (Borgarelli, et al., 2012). Concerning pre-clinical dogs, another study reported that 82% of them were still asymptomatic 12 months later (Moonarmart, et al., 2010). Many more studies can be found showing some minor variations between them (Borgarelli & Buchanan, 2012).

The differences amongst studies can be explained by inclusion of diverse criteria, different breeds, treatment and frequency of complications. Nonetheless, when taking into consideration all the studies, it can be concluded that patients with moderate heart failure due to DMVD receiving the appropriate medical treatment have fairly long clinical outcome, and the prognosis is relatively favorable for dogs with pre-clinical disease. Moreover, animals in asymptomatic stages tend to stay stable and not progress into heart failure when properly treated and monitored (Borgarelli & Buchanan, 2012). Further refinement of risk stratification schemes is needed to the development of a truly reliable method of evaluation, with good sensitivity and specificity (Keene, et al., 2019).

2.6 Clinical Signs and Physical Examination

There are usually no clinical signs in mild to moderate (ACVIM Stages A and B) cases of DMVD (Ljungvall & Häggström, 2016). In most dogs the disease is detected when a cardiac murmur is identified in a routine health care check-up or when managing a noncardiac illness (Abbott, 2016). This murmur is caused by mitral regurgitation, that is defined by is a systolic leakage of the mitral

valve. What causes the sound to which we call murmur is the blood flowing back from the left ventricle into the left atrium due to poor coaptation of the mitral leaflets (Chetboul, Bussadori, & Madron, 2016). Despite the fact that a systolic heart murmur is typically the most prominent clinical finding, a mid-systolic click may be heard in early stages of DMVD. It must be taken into account that the absence of an audible murmur cannot rule out mild regurgitation (Kvart & Häggström, 2002; Ljungvall, et al., 2009). Short physical stimulation, like a short run, can often augment the intensity of the murmur in early stages of the disease. In these patients the murmur may be intermittent and the point of its maximal intensity is on the left side of the thorax, over the mitral valve area (Ljungvall, Rishniw, Porciello, Ferasin, & Ohad, 2014; Reimann, et al., 2014). As the disease progresses, the intensity of the murmur augments as well as its duration, becoming gradually holosystolic and audible in both sides of the thorax (Ljungvall, et al., 2009). In patients with moderate or severe mitral regurgitation an exaggerated apical impulse on precordial palpation can be present, this impulse is usually referred to as precordial thrill (Abbott, 2016). The intensity of the heart murmur is of subjective evaluation but it can be classified as shown in table 1.

Table 1: Comparison of 4 level and 6 level murmur grading systems. Adapted from Rishniw, 2018.

4 level grading system	6 level grading system	Intensity of the murmur
Soft	I/VI and II/VI	Murmur is softer than the heart sounds
Moderate	III/VI	Murmur is equal to the heart sounds
Loud	IV/VI	Murmur is louder than the heart sounds
Palpable	V/VI and VI/VI	Murmur causes a palpable thrill

Cough is typically one of the first clinical signs reported by the owner. Dogs with left mainstem bronchial compression, but without pulmonary congestion or edema, may have a dry and harsh cough with coughing spells at any time during the day, more pronounced during physical exercise or excitement. In contrast, when coughing is caused by pulmonary congestion or edema it may be moist and productive, associated with tachypnea and dyspnea. However, recent evidence seems to oppose to the traditional dogmatic approach that related cough to congestive heart failure (CHF) in dogs (Ljungvall & Häggström, 2016; Ferasin & Linney, 2019). When a dog shows signs of CHF the pulmonary sounds are usually more perceptible with crackles, snaps and popping sounds, best heard at the end of inspiration. In animals with fulminant pulmonary edema, the expectoration of pink froth is sometimes observed (Ohad, Rishniw, Ljungvall, Porciello, & Häggström, 2013; Abbott, 2016; Ljungvall & Häggström, 2016).

The first clinical signs of decompensated CHF (ACVIM Stage C) are generally mild but can worsen over a period of days or weeks. Bearing in mind that these signs are non-specific, the diagnostic challenge is to establish whether the DMVD is responsible for them. Dogs in decompensated CHF

are often restless and anxious at nighttime and demonstrate a preference for lying in sternal recumbency. They can also be inactive and have different degrees of inappetence, with reducing resistance to exercise (Ljungvall & Häggström, 2016).

Sporadically, syncope is the clinical sign that is first seen in dogs with DMVD. Syncope is a transient loss of consciousness caused by an abrupt and quick decline in cerebral perfusion (Abbott, 2016). The event may be associated with tachyarrhythmia due to cardiac enlargement (Rasmussen, et al., 2011). Other causes of syncope include tussive fainting that may occur in conjunction with paroxysms of coughing or exercise in the presence of pulmonary hypertension (Rasmussen, et al., 2014; Ljungvall & Häggström, 2016). Less frequent clinical signs related to reduced cardiac performance are weak pulses and cyanotic mucous membranes (Ljungvall & Häggström, 2016).

Abdominal palpation is habitually normal in dogs with DMVD, nonetheless, when the right side of the heart becomes affected, hepatic and/or splenic enlargement can be felt or even ascites. In these cases, pulsations in the jugular veins may be present (Abbott, 2016; Ljungvall & Häggström, 2016).

2.7 Classification

Heart disease may or may not lead to heart failure, this progression depends not only on the nature of the disease and its rate of progression, but also on the patient's age and condition. Heart failure is a general term used to label a clinical syndrome caused by a variety of specific heart diseases, one of them being the DMVD. It refers to the clinical signs caused by heart dysfunction, and, regardless of the cause, it is always characterized by cardiac, hemodynamic, renal, neurohormonal, and cytokine abnormalities (Atkins, et al., 2009; Keene, et al., 2019).

When a cardiac disease starts to affect heart function beyond a certain point two situations can occur. On the one hand, venous pressures increase so severely that fluid accumulates in the lungs or in a body cavity [congestive heart failure (CHF)], and on the other hand, the heart's pumping ability is compromised in such a way that it cannot meet the body's needs either during exercise or at rest, in the face of either normal or increased venous pressure (Atkins, et al., 2009; Keene, et al., 2019).

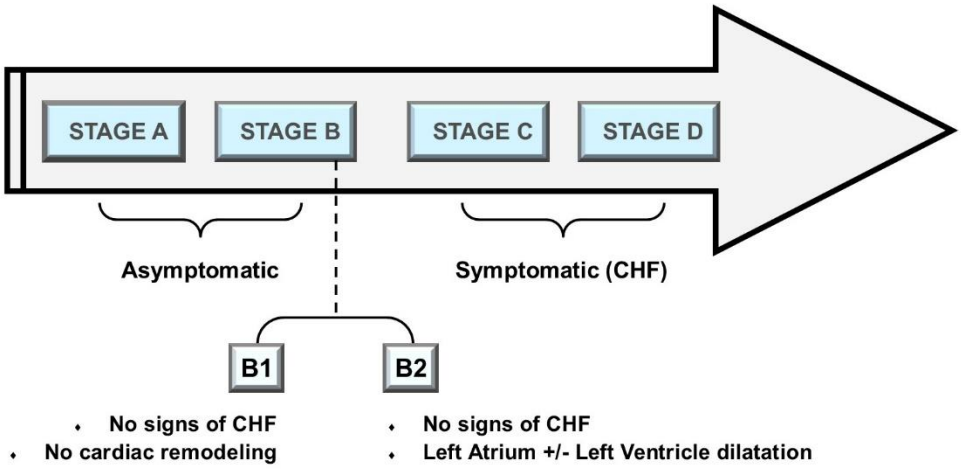
Many classification systems have been developed to better categorize the different stages of heart failure and, therefore, to provide a framework for discussing and comparing the clinical signs of patients in heart failure. Traditionally, the systems were based on the degree of functional limitation that results from cardiac disease. Examples include the New York Heart Association Functional Classification (NYHA) and a similar system proposed by the International Small Animal Cardiac Health Council. However, a big limitation of these systems is that they are poorly suited for the development of therapeutic guidelines, for it does not reflect the progressive nature of the heart failure state. Besides, they are based on relatively subjective assessments of clinical signs that can change frequently and dramatically over short periods of time (Atkins, et al., 2009; Abbott, 2016).

In 2009, the American College of Veterinary Internal Medicine (ACVIM) Specialty of Cardiology's consensus panel assembled and developed a newer classification system that better objectivizes the grading of patients in the course of their heart disease (Keene, et al., 2019). They made an adaptation of the 2001 American College of Cardiology/ American Heart Association classification system for heart disease and failure in human patients. The main goal was to associate the severity of morphologic changes and clinical signs to appropriate treatment at each stage of the illness. In this approach, unless the progression of the disease is altered by treatment, patients are expected to advance from one stage to the next. Even though this categorizing system remains useful, recent clinical trial results require a more critical clinical evaluation of dogs in Stage B to enable a sound therapeutic decision-making (Atkins, et al., 2009; Keene, et al., 2019).

There are 4 basic stages of heart disease and heart failure in this staging system for DMVD (see figure 6):

- Stage A: patients predisposed to the development of heart disease but that currently have no identifiable structural disorder of the heart (eg, every CKCS or other predisposed breed without a heart murmur).
- Stage B: patients that have structural heart disease (eg, murmur of mitral valve regurgitation, accompanied by some typical valve pathology), without clinical signs caused by heart failure associated.
    - Stage B1: asymptomatic dogs with no radiographic or echocardiographic evidence of cardiac remodeling in response to their DMVD.
    - Stage B2: asymptomatic dogs with radiographic and echocardiographic findings of left atrial and/or ventricular enlargement, compatible with a more hemodynamically severe and long-standing mitral valve regurgitation.
- Stage C: patients with structural abnormalities with current or previous clinical signs of heart failure caused by DMVD.
- Stage D: patients with end-stage DMVD, in which clinical signs of heart failure are refractory to standard treatment (Keene, et al., 2019).

Figure 6: American College of Veterinary Internal Medicine's classification system. Original based on Keene, et al., 2019.



2.8 Diagnosis of Degenerative Mitral Valve Disease

As stated before, DMVD is typically first detected during a routine health check-up by auscultation of a heart murmur caused by mitral regurgitation. In order to explore the initial clinical suspicion and to determine the hemodynamic relevance of the mitral defect, all patients should undergo a thoracic radiography examination. For the purpose of definitely identifying the cause of the heart murmur it is recommended the realization of an echography performed by an experienced operator (Keene, et al., 2019).

With the progression of the disease the signalment, the eventual history of clinical signs reported by the owners and the physical examination can lead the clinician to the diagnosis of heart failure caused by DMVD. The realization of a serum biochemical profile, a complete blood count and urinalysis is also a good idea, especially if treatment for CHF is anticipated. The need for these tests is justified by the frequent impaired renal function as a comorbidity in dogs with heart failure. Electrocardiography and measurement of bloodborne biomarkers may also proof helpful in some cases (Keene, et al., 2019).

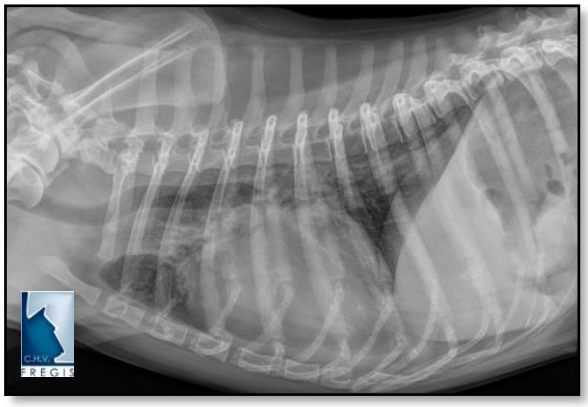
2.8.1 Radiographic Findings

Thoracic radiography constitutes an important tool to assess the hemodynamic consequences of DMVD and to possibly eliminate other causes for the clinical signs when present. Nonetheless, in asymptomatic dogs this complementary exam is also recommended so that a baseline can be established for the purpose of comparison in future health controls (Keene, et al., 2019).

The structures with paramount relevance for the diagnosis are the left atrium and ventricle, the mainstem bronchi, the pulmonary vessels and the lungs. In the early stages of DMVD, there are no alterations in the cardiac silhouette nor in the lung fields or in the vascular markings. However, when a significant amount of mitral regurgitation takes place, an enlargement of the heart can be expected.

One of the earliest and most constant radiographic features in DMVD is a left atrial enlargement. With the progression of the disease, the left ventricle starts to enlarge as well. In a lateral projection, the left atrium occupies the caudodorsal area of the cardiac silhouette. When the left atrium is enlarged a dorsal elevation of the caudal portion of the trachea and carina can be remarked (see figure 7), as well as straightened caudal border of the cardiac silhouette. If the enlargement is more significant, the left mainstem bronchus may be narrowed. In a ventrodorsal (VD) or dorsoventral (DV) projection the left atrium is close to the center of the cardiac silhouette, and, if enlarged, it can cause a protuberance in the left cranial part of the cardiac border (Abbott, 2016; Ljungvall & Häggström, 2016).

Figure 7: Lateral view radiography of a sixteen-year-old Mongrel dog with DMVD (ACVIM stage C). Original image, kindly provided by Dr. Eymeric Gomes from the Diagnostic Imaging Department of the Centre Hospitalier Vétérinaire Frégis.

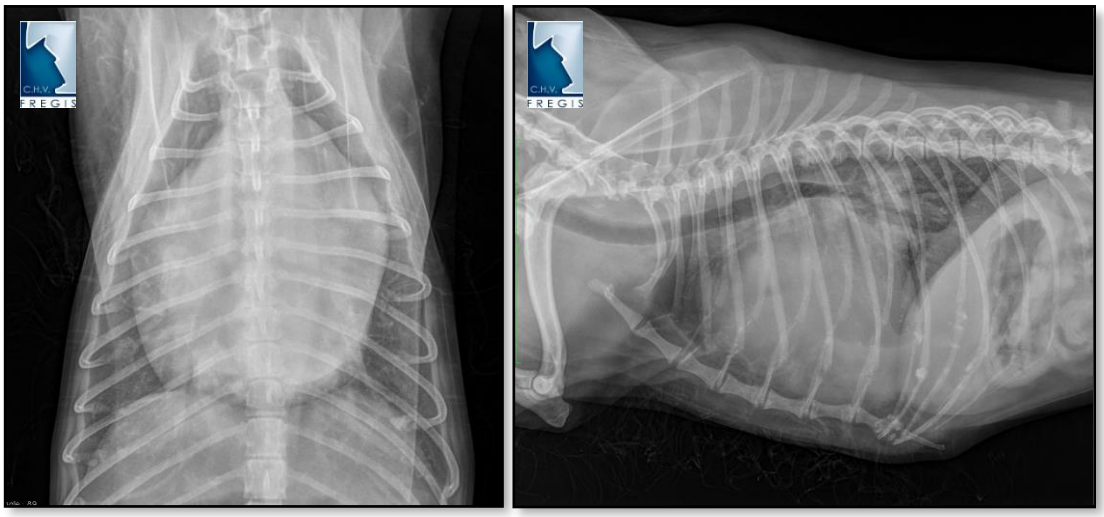


Legend: An enlarged heart and a dorsal elevation of the thoracic portion of the trachea can be observed.

An appreciation of the changes in the heart size can be made using the Vertebral Heart Score (VHS) and the Vertebral Left Atrial Size (VLAS) (Malcom, Visser, Philips, & Johnson, 2018). The VHS tends to rise in a slow and steady manner until about 6 to 12 months before the onset of CHF, after that period of time the rate of change becomes quicker (Lord, et al., 2010). It is important to bear in mind the marked variations in thoracic conformation and breed differences in the normal VHS when analyzing this parameter (Keene, et al., 2019).

As the DMVD progresses radiographic signs of pulmonary venous distention may be present, in this scenario the diameter of the veins is bigger than that of the corresponding arteries. This distention is suggestive of pulmonary congestion which normally precedes the development of pulmonary edema, which can blur the vascular structures. In dogs, the cardiogenic pulmonary edema has often a perihilar distribution (see figures 8 and 9), sometimes more noticeable on the right side and in the dorsal aspect of the caudal lung lobes. Acute edema can, however, affect the cranial lobes (Abbott, 2016; Ljungvall & Häggström, 2016). If air bronchograms are seen, it is probably due to fluid entering the pulmonary alveoli which provides contrast with the structures filled with air, signifying that the edema evolved from interstitial to alveolar. A radiography with signs of both left atrial enlargement and pulmonary edema enables the clinician to make a diagnosis of CHF (Abbott, 2016).

Figure 8 and Figure 9: Dorsoventral and lateral view radiographs of a nine-year-old Cavalier King Charles Spaniel with DMVD (ACVIM stage C). Original image, kindly provided by Dr. Eymeric Gomes from the Diagnostic Imaging Department of the Centre Hospitalier Vétérinaire Frégis.



Legend: A severe cardiomegaly can be observed in both radiographs as well as a symmetric perihilar opacification of the pulmonary parenchyma indicating pulmonary edema. In the lateral view a dorsal elevation of the thoracic portion of the trachea is visible.

2.8.2 Electrocardiographic Findings

The electrocardiographic tracings of animals with DMVD may be normal or show marked abnormalities in rate, rhythm or configuration of the complexes (Ljungvall & Häggström, 2016). The main interest of the electrocardiogram (ECG) is to document and classify arrhythmias, nonetheless, it can provide indirect evidence of chamber enlargement (Abbott, 2016; Ljungvall & Häggström, 2016).

In the early stages of DMVD the sinus arrhythmia is usually preserved. When the patient evolves to a stage where CHF is present, this sinus arrhythmia can be loss and a sinus tachycardia can appear. Arrhythmias are one of the complications of DMVD, and most often present as supraventricular premature beats (Crosara, et al., 2010; Rasmussen, et al., 2014). Atrial fibrillation, paroxysmal supraventricular tachycardia, atrioventricular dissociation, ventricular premature beats and ventricular tachycardia can also occur but are less common and normally encountered in animals in advanced stages of the disease. As a result, they are an indicator of poor prognosis (Ljungvall & Häggström, 2016).

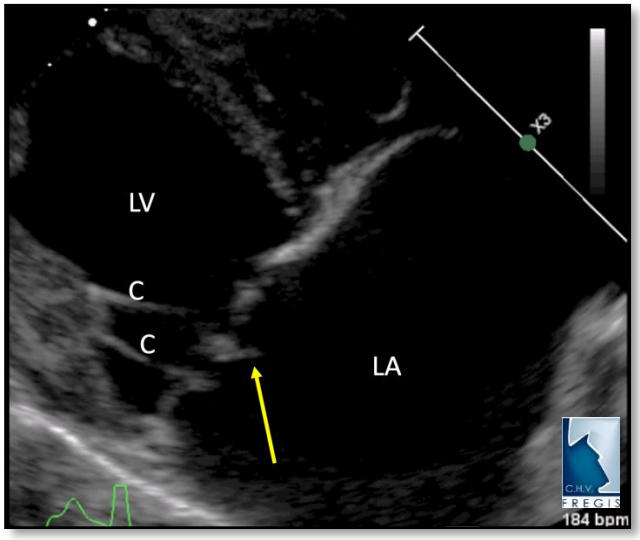
As far as the cardiac chamber size is concerned, ECG is an insensitive indicator of heart enlargement and cannot detect CHF. Throughout the evolution of DMVD, the electric axis in the frontal plane frequently stays within normal range (Ljungvall & Häggström, 2016). A P wave with increased duration (*P mitrale*) or increased amplitude may be present whenever there is an important enlargement of the left atrium. Moreover, in the case of significant left ventricle enlargement, the QRS complex may also be prolonged and the amplitude of the R wave in lead II augmented (Oyama, Kraus, & Gelzer, 2014).

2.8.3 Echocardiographic Findings

An echocardiography performed by an experienced operator can confirm the presence of valvular regurgitation, detect valve lesions, record cardiac remodeling, estimate intracardiac pressures and quantify systolic ventricular function (Bonagura & Schober, 2009). Furthermore, it is the best way to determine if a murmur is caused by DMVD of not (Keene, et al., 2019). Hence, the importance of this exam in obtaining a diagnosis, monitoring the condition, establishing a prognosis and selecting a medical therapy (Bonagura & Schober, 2009; Keene, et al., 2019). However, when used to predict the presence of CHF, even if some echocardiographic variables are useful they are not more accurate or sensitive than other clinical tests like, for instance, measuring of the respiratory rate (Schober, et al., 2010).

The evaluation of the mitral valve's anatomy and identification of leaflet thickening and systolic leaflet prolapse can be done in a two-dimensional (2-D) echocardiography (Bhave & Lang, 2011). Regarding the mitral valve prolapse and the degenerative changes of the leaflets, their presence and severity are best analyzed in the right parasternal long-axis view and the affected tissue generally has an uniform echogenicity (Borgarelli, et al., 2012; Abbott, 2016). When the edge of the leaflet or, in more severe cases, the entire leaflet moves into the LA during systole, it is usually due to the rupture of a *chordae tendineae*, which may be visible in the LA or LV (see figure 10). The flail motion of the valve leaflet and the chordal thickening are best observed in the right parasternal long-axis view and in the left apical four-chamber view, in a plane parallel to the long axis of the left-side heart chambers (Jacobs, Calvert, Mahaffey, & Hall, 1995).

Figure 10: Ultrasonographic image showing the right parasternal long-axis view of a twelve-year-old Lucas Terrier. Original image, kindly provided by Dr. Cecile Damoiseaux from the Cardiology Department of the Centre Hospitalier Vétérinaire Frégis.

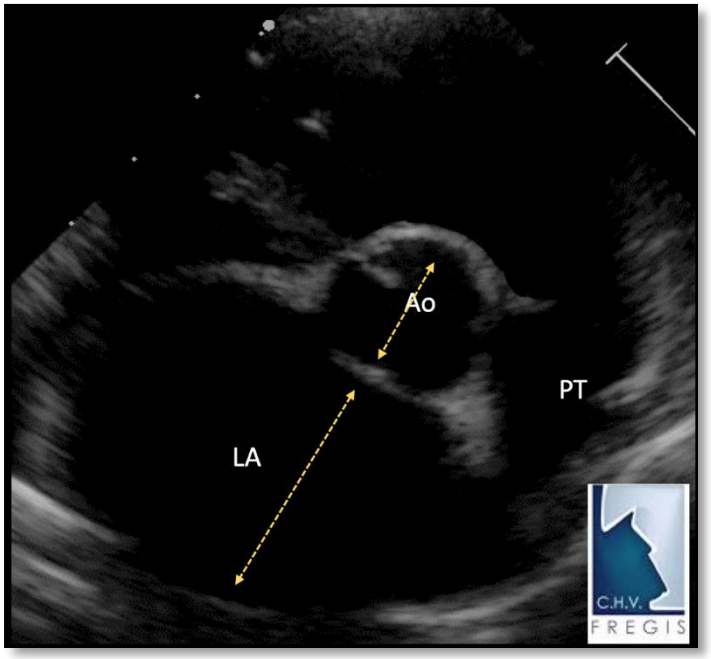


Legend: The yellow arrow points to a ruptured principal *chorda tendinea* (C) of the anterior mitral leaflet prolapsing into the dilated left atrium (LA). Moreover, thickening of the mitral apparatus can be observed. LV stands for left ventricle.

As far as the LA size is concerned, it can be examined from different views. Regardless, it is important to have an anatomic reference relatively constant for a given dog size in order to best assess the presence or absence of LA enlargement. Consequently, the finest view to do so is the right parasternal short-axis view, where the LA body, the aortic root (Ao) and the auricle are visible (see figure 11), since the ratio LA/Ao is a great way to identify an augmentation in the LA size (Hanson, Häggström, Kvart, & Lord, 2002). Moreover, one of the criteria decisive to beginning the treatment in ACVIM Stage B dogs is having, in early diastole, a ratio superior or equal to 1.6 (Keene, et al., 2019).

LV anatomical dimensions, volume and function can also be evaluated in both one-dimensional (M-mode) and 2-D images (Meyer, et al., 2013). Even though it has been demonstrated that assessing the LV volume can be interesting in dogs with DMVD, breed-specific normal reference ranges are currently only available to a few breeds (Ljungvall & Häggström, 2016). With respect to the LV size, as said before, it tends to progressively enlarge with the evolution of the disease, presenting a rapid increase in size during the last year before the onset of CHF, thus suggesting that the rate of increase in cardiac dimensions can be an indicator of imminent decompensation (Lord, et al., 2010; Ljungvall, Höglund, Carnabuci, Tidholm, & Häggström, 2011).

Figure 11: Ultrasonographic image showing the right parasternal transaortic short-axis view of a six-year-old Cavalier King Charles Spaniel. Original image, kindly provided by Dr. Cécile Damoiseaux from the Cardiology Department of the Centre Hospitalier Vétérinaire Frégis.

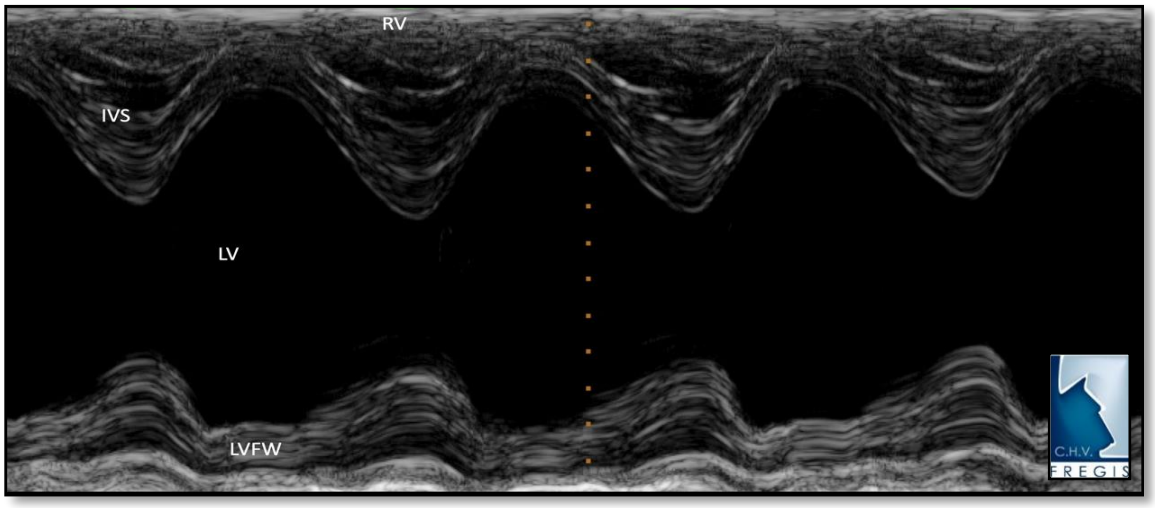


Legend: An enlarged left atrium (LA) body compared to the aortic root (Ao) can be observed. In this case the LA/Ao ratio is 2.0. PT stands for pulmonary trunk.

Although difficult in DMVD patients, identification of systolic dysfunction may be done using echocardiographic modalities (see figure 12). The challenge in doing so comes from the fact that the

echocardiographic indices used for evaluating systolic function, such as ejection phase indices, are not only dependent on intrinsic contractility but also influenced by hemodynamic load and sympathetic tone. Consequently, dogs with mitral regurgitation may have a masked significant myocardial dysfunction (Bonagura & Schober, 2009). Nonetheless, it has been suggested that the assessment of the LV end-systolic function better reflects systolic dysfunction in the presence of mitral regurgitation (Borgarelli, Tarducci, Zanatta, & Häggström, 2007). When the systolic function deteriorates, in spite of the increasing retrograde LV stroke volume into the LA, the end-systolic dimension or volume increases. However, an almost normal end-systolic dimension is expected if the LV contractile function is preserved by compensatory mechanisms (Bonagura & Schober, 2009).

Figure 12: Ultrasonographic image showing the M-mode of the right parasternal transaortic short-axis view of a six-year-old Cavalier King Charles Spaniel. Original image, kindly provided by Dr. Cécile Damoiseaux from the Cardiology Department of the Centre Hospitalier Vétérinaire Frégis.



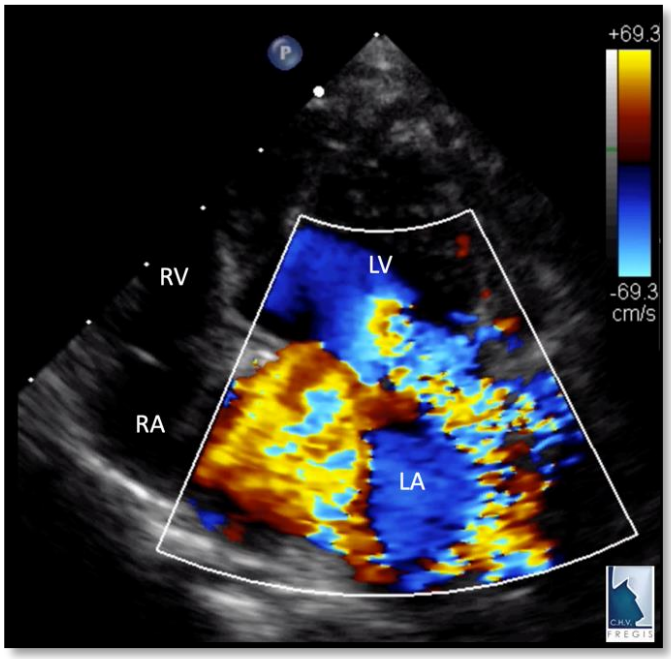
Legend: M-mode showing an increase of the left ventricular (LV) end-diastolic diameter (46.0 mm) when compared to the reference value for the breed. IVS stands for interventricular septum, LVFW for left ventricular free wall and RV for right ventricle.

In order to detect and quantify the mitral regurgitation, spectral and color-flow Doppler are the best tools. On the one hand color-flow Doppler echocardiography confirms the presence of a regurgitant jet, on the other hand spectral Doppler gives information on its velocity. The best view to correctly evaluate the regurgitation is the left apical four-chamber view, since the regurgitant flow should be aligned with the ultrasound beam (Ljungvall & Häggström, 2016).

Evaluation of mitral regurgitation severity is extremely important in the presence of DMVD, since mitral regurgitation directly reflects the primary hemodynamic consequence of imperfect apposition of the mitral leaflets during systole. This evaluation can be done by semi-quantification or quantification of the regurgitant jet (Chetboul & Tissier, 2012). Using the color-flow Doppler mode (see figure 13), the maximal ratio of the regurgitant jet area signal to the LA area can be calculated, and represents one frequently used method to assess mitral regurgitation severity (Muzzi, de Araújo, Muzzi, Pena, & Silva, 2003). Following the execution of this method, the mitral regurgitation is

considered mild if the ratio is inferior to 20-30%, moderate if it is around 20-30% and severe when superior to 70% (Zoghbi, et al., 2003). This color mapping technique is only semi-quantitative given the fact that two areas are being compared without any assessment of the regurgitant volume. Nonetheless, it is a method of rapid execution that permits the acquisition of data with ease, with good repeatability and reproducibility in the awake dog for a trained observer (Gouni, et al., 2007). In order to quantify the mitral regurgitation, the Proximal Isovelocity Surface Area (PISA) method, also called flow convergence method, can be used (Zoghbi, et al., 2003). It provides a measurement of the flow rate through the regurgitant orifice, calculates the regurgitant volume, and assesses the regurgitation fraction which is the percentage of stroke volume ejected into the LA during systole. Even though it is a more time-consuming technic than color mapping and requires more expertise to well-execute, it is has also shown to be repeatable and reproducible in the awake dog for a trained observer. Mitral insufficiency is normally considered as moderate when the regurgitation fraction values are higher than 30-50% and severe if higher than 70% (Kittleson & Brown, 2003; Gouni, et al., 2007).

Figure 13: Ultrasonographic image showing the left apical four-chamber view of a six-year-old Cavalier King Charles Spaniel. Original image, kindly provided by Dr. Cecile Damoiseaux from the Cardiology Department of the Centre Hospitalier Vétérinaire Frégis.



Legend: An holosystolic mitral regurgitation detectable with color-flow Doppler which occupies 100% of the left atrium (LV) area. RA stands for right atrium and RV for right ventricle.

2.8.4 Bloodborne Biomarkers

Due to the non-specific nature of DMVD clinical signs, differentiating between respiratory distress caused by CHF or by primary respiratory tract disease can be challenging when there is no access to echocardiography. As a consequence, a blood-based assay that allows this differentiation can be useful (Oyama, et al., 2009).

B-type natriuretic peptide (BNP) and A-type natriuretic peptide (ANP) along with their parent proteins proBNP and proANP respectively, are the main natriuretic hormones produced by the myocardial tissue. Both proBNP and proANP are released by atrial and ventricular cardiomyocytes in response to stress or stretch of the myocardium, for instance as a consequence of volume overload. Once released, these proteins are rapidly cleaved into separate N-terminal and C-terminal fragments. N-terminal pro-BNP (NT-proBNP) and N-terminal pro-ANP (NT-proANP) are the most stable and make more attractive targets for assay. Hence, their levels can be measured with commercially available tests, and it was demonstrated that dogs with clinical signs caused by a cardiac abnormality have higher serum levels than those with a non-cardiac disease (Oyama, Fox, Rush, Rozanski, & Lesser, 2008; Oyama, et al., 2009) Consequently, normal concentration levels of NT-proBNP in an animal with cough, dyspnea, or exercise intolerance is highly suggestive of a non-cardiac cause for the clinical signs (DeFrancesco, et al., 2007; Oyama, et al., 2009). Furthermore, NT-proBNP concentrations are positively correlated with radiographic and echocardiographic measures of disease severity, which means that dosing this substance has both diagnostic and prognostic value. NT-proBNP is particularly useful in ruling out CHF, since patients with low or normal values of this substance are highly unlikely to have CHF. Regarding its value in predicting the first onset of CHF, it is best when combined with radiographic or echocardiographic left side heart measures (Oyama, 2015).

Another cardiac biomarker is the cardiac troponin. Damage to the cardiomyocytes and to the sarcolemmal membrane dissociates troponin from the actin and results in the leakage of troponin into the extracellular space where it enters in circulation. Circumstances that may warrant troponin testing include prognostic information retrieval in DMVD patients. Generally, cardiac troponin concentrations reflect the severity of cardiac injury and are inversely related with morbidity and mortality (Ljungvall, et al., 2010).

It is, nonetheless, important to point out that cardiac biomarker tests are complementary to other diagnostic exams and should, at all times, be interpreted in the context of the overall clinical picture (Oyama, 2015).

2.8.5 Other Considerations

For ACVIM Stage A animals, since there are yet no identifiable structural disorders it is important to try to detect the disease as soon as it appears. Therefore, it is recommended regular medical evaluations in which a cardiac auscultation should be performed. Additionally, owners of dogs at particularly high risk, suchlike CKCS, are encouraged to partake in yearly screening events conducted by board-certified cardiologists (Keene, et al., 2019).

2.9 Treatment of Degenerative Mitral Valve Disease

To this day, there is no medical cure for DMVD. The lack of knowledge about the factors that cause the disease has prevented the development of effective long-term clinical management plans. In the

absence of a cure, the medical treatment is primarily directed at managing and delaying the clinical signs. Even though a surgical repair or replacement of the mitral valve is possible, it requires a high level of expertise to be successful, as well as an elevated financial capacity of the owner. Consequently, its applicability to the general population is limited (Gordon, et al., 2017). It is, however, important to bear in mind that there are certain stages of the disease to which there is disagreement within the medical community regarding what treatment should be administered (Keene, et al., 2019). In this dissertation, only the recommendations that are agreed on by most of the community will be mentioned.

2.9.1 ACVIM Stage A and B1

Concerning the animals in ACVIM Stage A (patients at high risk of developing heart failure but that currently have no identifiable structural disorder of the heart) no pharmacologic or dietary treatment is recommended. However, young breeding animals that develop a murmur should not be bred anymore (Keene, et al., 2019). The same is true for animals in ACVIM Stage B1 (asymptomatic dogs with no radiographic or echocardiographic evidence of cardiac remodeling), given the fact that at such an early stage of disease the patient is not certain to progress to heart failure. Furthermore, it seems to exist no evidence to support the efficiency of a dietary or drug treatment at this point. It is, nonetheless, recommended a reevaluation by ultrasonography and/or radiography in a six to twelve months' time. A more frequent follow-up is suggested for large dogs (Keene, et al., 2019).

2.9.2 ACVIM Stage B2

When the mitral valve regurgitation becomes long-standing and more hemodynamically severe, the result of the heart's compensating mechanisms starts to be visible in radiographic and echocardiographic findings (Keene, et al., 2019). Animals in this stage of the disease are graded as ACVIM B2 and, according to the 2019 ACVIM consensus guidelines, in order for a treatment to be beneficial there are multiple criteria that ought to be met:

1) heart auscultation should reveal a murmur intensity of 3/6 or higher (Keene, et al., 2019);

2) the ratio between the diameters of the left atrium and the aorta (LA/Ao) in early diastole should be at least 1.6, this measure can be obtained in the right-sided short axis view in an echocardiography (Hanson, et al., 2002);

3) when in diastole the left ventricular diameter, normalized for body weight, should be equal or superior to 1.7 (Cornell, et al., 2004);

4) the breed-adjusted radiographic vertebral heart score (VHS) should be 10.5 or higher (Lamb, Wikeley, Boswood, & Pfeiffler, 2001; Bavegems, et al., 2005).

Once these criteria are met, a treatment with pimobendan should be initiated at a dose of 0.25-0.3 mg/kg *per os* (PO) every 12 hours (Boswood, et al., 2016; Boswood, et al., 2018). It is also recommended to adapt the diet so that it has a reduced sodium content and a highly palatable taste, with the right amount of protein and calories to maintain an ideal body condition (Freeman, Rush, & Markwell, 2006). Amongst the criteria above cited, the echocardiographic sign of left atrial and

ventricular enlargement is the most consistent way to assess that a dog is going to benefit from treatment (Keene, et al., 2019).

2.9.3 ACVIM Stage C and D

As soon as a dog exhibits clinical signs of heart failure it is necessary to modify the treatment plan. For both ACVIM Stage C and D patients there is a significant difference between being in acute heart failure in need of hospital care and being in a more chronic phase that is manageable from home. As far as acute heart failure is concerned, the objective is to normalize the patient's hemodynamic status and tissue oxygen delivery so that the cardiac output can be enhanced, the mitral valve regurgitation reduced and the clinical signs relieved. Regarding the chronic management, the goal is not only to maintain the hemodynamic status regularized, but also to slow the progression of the disease, to extend life expectancy without jeopardizing its quality, to augment physical exercise resistance, to reduce clinical signs and to maintain an optimal body weight (Keene, et al., 2019).

Animals with heart failure tend to lose muscle or lean body mass, a phenomenon called cardiac cachexia that may or may not be accompanied by clinically relevant weight loss. The prognosis of dogs with cachexia, in comparison with those with a good body condition, is worse. Consequently, a dietary treatment is an important prevention tool, given the fact that preventing cardiac cachexia is much easier than treating it (Freeman, 2011). For this reason, an adequate calorie intake should be maintained, as well as a record of the patient's body condition score and accurate weight. In addition, the diet should have an appropriate protein content (low-protein diets should be avoided) and be modestly restricted in sodium (Keene, et al., 2019). Omega-3 fatty acids can be added, notably in dogs with muscle loss, reduced appetite and arrhythmias (Freeman, et al., 1998). Monitoring the serum concentrations of potassium and magnesium is recommended, so that a proper supplementation can be added if needed. These recommendations are applicable to both ACVIM Stage C and D patients (Keene, et al., 2019).

2.9.3.1 ACVIM Stage C

ACVIM Stage C patients present structural abnormalities with current or previous clinical signs of heart failure caused by DMVD. When a hospital-based treatment is required, these animals should receive furosemide at a dose of 2 mg/kg administered via intravenous (IV) or intramuscular (IM) followed by 2 mg/kg IV or IM every hour until the respiratory signs subside or a total dose of 8 mg/kg has been reached over 4 hours. Once the diuresis has begun, the patient should be allowed free access to water. Pimobendan at a dose of 0.25-0.3 mg/kg PO every 12 hours should also be given to the dog. If needed, oxygen supplementation should be provided as well as sedation with narcotics or a narcotic compound combined with an anxiolytic compound, in the case of an anxious animal with dyspnea. An optimal nursing care is imperative, which means that an ideal environmental temperature and humidity should be preserved, the head should be kept higher than the rest of the body with pillows, and sedated patients should be placed in a sternal posture. When the patient fails to respond adequately to diuretics, pimobendan, sedation, oxygen and comfort care measures,

dobutamine may be used in addition to improve ventricular function at a dose of 2.5-10 μg/kg/min as a Continuous Rate Infusion (CRI), starting at 2.5 μg/kg/min and increasing the dose gradually (Keene, et al., 2019).

Once the treatment can be done at home, furosemide PO at the dose of 2 mg/kg every 12 hours should be administered, with the possibility of augmenting the frequency of administration if the dog shows discomfort (Keene, et al., 2019). Pimobendan at the dose of 0.25-0.3mg/kg PO every 12 hours should also be continued (Häggström, et al., 2008). It is recommended to administer an Angiotensin Converting Enzyme Inhibitor (ACEI), for example enalapril or benazepril at a dose of 0.5mg/kg PO every 12 hours, or an equivalent dose of another ACEI indicated for this use (Keene, et al., 2019). Spironolactone (0.2 mg/kg PO every 12-24 hours) should also be administered (Ames, Atkins, Eriksson, & Hess, 2017). A surgical repair of the mitral apparatus can be beneficial when performed in veterinary centers with low complication rates (Mizui, Mizukoshi, & Uechi, 2013). In addition to the treatments, owners should surveil the respiratory and heart rate, and the appetite (Keene, et al., 2019). An increased resting respiratory rate (>40/minute) is highly suggestive of an impending clinical decompensation (Porciello, et al., 2016). For animals with concurrent persistent atrial fibrillation, it is recommended the addition of digoxin, generally in combination with diltiazem. Cough suppressants and bronchodilators may prove advantageous in some ACVIM Stage C patients (Keene, et al., 2019).

2.9.3.2 ACVIM Stage D

Animals labeled as ACVIM Stage D have clinical signs of heart failure that are refractory to standard treatments for ACVIM Stage C patients. For this reason, they require more than 8 mg/kg of furosemide daily, administered simultaneously with the other medications recommended in ACVIM Stage C, namely pimobendan, spironolactone and an approved ACEI (Keene, et al., 2019).

When hospitalized, and given that the dog has no severe renal insufficiency, an additional 2mg/kg IV bolus of furosemide followed by either more bolus doses or a CRI at a dose of 0.66-1 mg/kg/h can be given to dyspneic patients until the normalization of the respiratory function is achieved, or for a maximum of 4 hours (Keene, et al., 2019). In alternative, to treat animals no longer responsive to furosemide, torsemide can be administered at a dose of 0.1-0.2 mg/kg every 12 or 24 hours or approximately 5-10% of the furosemide dose (Peddle, et al., 2012). Additionally, a cavitary centesis can be performed if needed to alleviate the respiratory distress and discomfort. In the presence of clinically relevant pulmonary hypertension, giving sildenafil at a dose of 1-2 mg/kg every 8 hours is recommended. The dose of pimobendan may be increased to include a third 0.3mg/kg daily PO dose, it is, however, an off-label use (Keene, et al., 2019).

Once in a chronic phase of DMVD, the treatment from home is similar to the one of chronic ACVIM Stage C patients. The different aspects are the possible off-label increase of the dose of pimobendan and the changing to torsemide when the response to furosemide is no longer satisfactory, at a dose of 0.1-0.2 mg/kg every 12 or 24 hours or approximately 5-10% of the furosemide dose, up to 0.6

mg/kg every 12 hours if necessary (Oyama, Peddle, Reynolds, & Singletary, 2011; Keene, et al., 2019). Hydrochlorothiazide is also recommended as a complementary treatment to furosemide or torsemide. Similarly, to the acute ACVIM Stage D treatment, when a clinically relevant pulmonary hypertension is present, sildenafil is recommended at the same dose (Keene, et al., 2019). The DMVD's treatment is schematized in the table 2.

Table 2: Degenerative Mitral Valve Disease's treatment according to American College of Veterinary Internal Medicine's guidelines. Original based on Keene, et al., 2019.

Stage	Description	Treatment
A	Patients at high risk of developing heart failure	**No treatment**
B1	No clinical signs of CHF No cardiac remodeling	**No treatment**
B2	No clinical signs of CHF Left Atrium +/- Left Ventricle dilatation	▪ **Pimobendan** (2 mg/kg PO q12h) ▪ **Appropriate dietary management**
C	Acute CHF	▪ **Furosemide** (2 mg/kg IV or IM followed by 2 mg/kg IV or IM every hour until improvement of respiratory signs or a total of 8 mg/kg over 4h) ▪ **Pimobendan** (0.25-0.3 mg/kg PO q12h) ▪ **Oxygen supplementation** ▪ **Sedation with narcotics/ narcotic compounds + anxiolytic compound** (anxious dyspneic animal) ▪ **Optimal nursing care** ▪ **Dobutamine** (2.5-10 µg/kg/min CRI, starting at 2.5 µg/kg/min and increasing the dose gradually) ▪ **Appropriate dietary management**
	Chronic CHF	▪ **Furosemide** (2 mg/kg PO q12h) ▪ **Pimobendan** (0.25-0.3 mg/kg PO q12h) ▪ **ACEI (**dose recommended by the laboratory according to the molecule) ▪ **Spironolactone** (0.2 mg/kg PO q12-24h) ▪ **Digoxin**, **Diltiazem, cough suppressants, bronchodilators** if needed ▪ **Appropriate dietary management**
D	Acute CHF refractory to treatment	Equal to Acute and Chronic Stage C plus: ▪ **Furosemide** (additional 2mg/kg IV bolus + more bolus doses or CRI 0.66-1 mg/kg/h until improvement of respiratory signs or a maximum of 4h) ▪ **Torsemide** (0.1-0.2 mg/kg q12-24h or 5-10% of the furosemide dose) in alternative to furosemide ▪ **Pimobendan** (0.3 mg/kg PO q8h – off-label use) ▪ **Sildenafil** (1-2 mg/kg q8h in case of pulmonary hypertension)
	Chronic CHF refractory to treatment	Equal to Chronic Stage C plus: ▪ **Torsemide** (0.1-0.2 mg/kg q12-24h or 5-10% of the furosemide dose) in alternative to furosemide ▪ **Pimobendan** (0.3 mg/kg PO q8h – off-label use) ▪ **Hydrochlorothiazide** ▪ **Sildenafil** (1-2 mg/kg q8h in case of pulmonary hypertension)

3. Specific measures on thoracic radiographs

3.1 Introduction

All dogs suspected of having DMVD, with or without clinical signs, are recommended to undergo a thoracic radiographic examination (Keene, et al., 2019). In order to proper diagnose and assess the severity of cardiac disease, the evaluation of the size and shape of the cardiac silhouette on radiographs is essential (Buchanan, 2000). In comparison to echocardiography, radiography of the thorax is widely available and cost-effective. However, marked variations in thoracic conformation amongst dog breeds difficult the interpretation of cardiac measures which determine heart enlargement. Furthermore, inconsistent positioning for radiography, the phase of the respiratory or cardiac cycles and concomitant non-cardiac thoracic affections can also lead to inaccurate evaluations of the size of the heart, especially when made by inexperienced observers (Silverman & Suter, 1975; Ruehl & Thrall, 1981; Holmes, Smith, Lewis, & Kern, 1985; Toal, Losonsky, Coulter, & De Novellis, 1985).

For example, when looking at lateral radiographs, a normal sized heart should measure the equivalent of 2,5 to 3,5 intercostal spaces for deep- or wide-chested dogs, respectively. Even though it is a simple way to determine an eventual cardiomegaly, this method has many limitations, namely variations in the cardiac axis, phase of respiration, chest shape, superimposition of ribs, and inexact measurement points. For the purpose of overcoming these limitations, cardiothoracic ratios were investigated with the objective of establishing a method that is precise, repeatable, simple to use and explain, and that, therefore, maximizes the accuracy of radiographic diagnosis. Due to a good correlation between heart weight and body length, regardless of the conformation of the thorax, emphasis was placed on comparisons of heart size and vertebral length (Buchanan & Bücheler, 1995; Buchanan, 2000).

3.2 VHS – Vertebral Heart Score

The VHS was first described by Buchanan and Bücheler in 1995 as a useful method to evaluate the size of the heart in dogs. The VHS is particularly interesting for inexperienced observers who are more prone to false positive interpretations when examining radiographs of brachycephalic breeds, obese dogs or puppies, given the fact that these patients normally present a rounder cardiac silhouette that bears resemblance to an enlarged heart (Buchanan, 2000).

The first step of the method consists in measuring, with a caliper, the long axis of the heart in lateral thoracic radiographs. The long axis of the cardiac silhouette is measured from the ventral border of the left mainstem bronchus to the most distant ventral contour of the apex, and reflects the combined size of the left atrium and ventricle. The caliper should then be repositioned over thoracic *vertebrae*, starting at the cranial edge of T4, to see how many *vertebrae* fall within the caliper points (Buchanan, 2000).

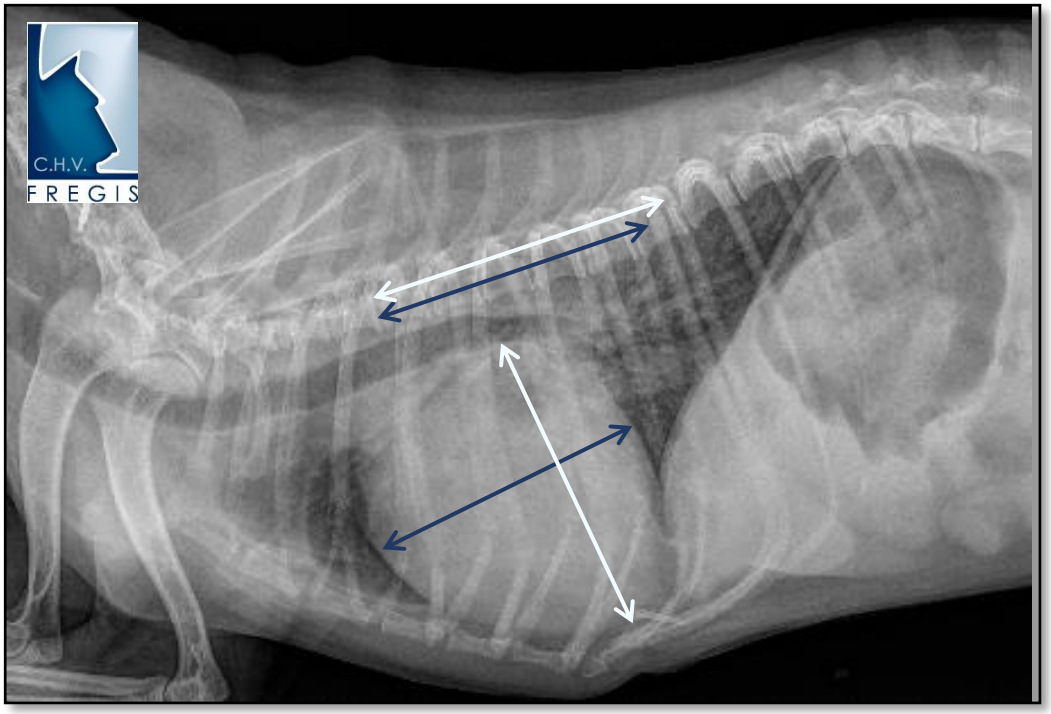
The next step is to measure the short axis in the central third region, at the widest part of the cardiac silhouette, perpendicular to the long axis. After this is done, the caliper should be transferred to the *vertebrae*, once again starting at the cranial edge of the T4 to count the number of *vertebrae* within the caliper points (see figure 14) (Buchanan, 2000).

Lastly, the short and long axis dimensions are added to obtain a *vertebrae*: heart sum, expressed as total units of vertebral-body length to the nearest 0,1 vertebra (Buchanan, 2000).

Considering the possibility of not having a caliper, the alternative is to use a sheet of paper. To measure the long axis a corner of the sheet should be placed at the base of the heart and then marked at the apex. Afterwards, in order to obtain the short axis measure, the same corner should be placed at the cranial edge of the heart and the caudal border marked. This corner is then transferred to the cranial edge of T4 so that the measures can be converted to number of *vertebrae* between the corner and the marked axes. Adding these values will result in the VHS (Buchanan, 2000). Nowadays, informatic programs come with a digital caliper that simplifies and accurately measures the axes above mentioned.

These measures can also be done in DV or VD projections but the results are more variable. When doing so, DV radiographs are preferred over VD ones, given the fact that cardiac contours are more consistent in DV projection and because there is magnification in VD radiographs due to a greater distance between the heart and the cassette (Buchanan, 2000).

Figure 14: Lateral radiographic projection of an 11-year-old dog demonstrating the VHS method. Original image, kindly provided by Dr. Eymeric Gomes from the Diagnostic Imaging Department of the Centre Hospitalier Vétérinaire Frégis



In Buchanan and Bücheler's study it was determined that the mean VHS on lateral radiographs for 100 normal dogs of several breeds was 9.7 +/- 0.5 *vertebrae* (Buchanan & Bücheler, 1995). However, the different conformation of the different breeds proves once again to be a limitation, since any method of measurement with a normal range wide enough to include the variations of different dogs and breeds is bound to be insensitive. Numerous breeds have been reported to have higher VHS mean values. For instance, a normal Boxer has considerably higher VHS mean values, as well as Labrador retrievers, CKCS and Whippets, amongst others (Lamb, et al., 2001; Bavegems, et al., 2005). As a consequence, it is more accurate to use breed-specific normal ranges when analyzing this parameter, like those indicated in Table 3.

Table 3: Breed-specific VHS mean values measured on right lateral recumbency

Dog Breed	Breed-specific VHS	Reference
Boxer	10.3 – 12.6	(Lamb, et al., 2001)
Labrador Retriever	9.7 – 11.7	(Lamb, et al., 2001)
German Shepherd dog	8.7 – 11.2	(Lamb, et al., 2001)
Doberman	9.0 – 10.8	(Lamb, et al., 2001)
CKCS	9.9 – 11.7	(Lamb, et al., 2001)
Yorkshire Terrier	9.0 – 10.5	(Lamb, et al., 2001)
Poodles	11.0 ± 0.5	(Pinto & Iwasaki, 2004)
Whippets	10.12	(Bavegems, et al., 2005)
Greyhound	10.5 ± 0.1	(Marin, et al., 2007)
Rottweilers	9.8 ± 0.1	(Marin, et al., 2007)
Beagle	10.5 ± 0.4	(Kraetschmer, Ludwig, Meneses, Nolte, & Simon, 2008)
Pug	10.7 ± 0.9	(Jepsen-Grant, Pollard, & Johnson, 2013)
Pomeranian	10.5 ± 0.9	(Jepsen-Grant, et al., 2013)
Bulldog	12.7 ± 1.7	(Jepsen-Grant, et al., 2013)
Shih Tzu	9.5 ± 0.6	(Jepsen-Grant, et al., 2013)
Lhasa Apso	9.6 ± 0.8	(Jepsen-Grant, et al., 2013)
Boston Terrier	11.7 ± 1.4	(Jepsen-Grant, et al., 2013)
Spitz	10.21 ± 0.23	(Bodh, et al., 2016)
Dachshund	10.3	(Birks, et al., 2017)

The principal use of the VHS method is to assess the presence of cardiomegaly in borderline cases, along with being able to do a quantification of the progression of heart enlargement over time in a given case (Buchanan, 2000). Furthermore, according to the experts of the ACVIM consensus, a breed-adjusted VHS measurement superior to 10.5 enables not only the assessment of cardiomegaly but also the classification of a dog in ACVIM B2 stage (Keene, et al., 2019).

3.3 VLAS – Vertebral Left Atrium Size

The magnitude of the LA enlargement is a reliable indicator to assess not only the severity of DMVD and the hemodynamic status of the patient but also the prognosis (Borgarelli & Häggström, 2010; Borgarelli, et al., 2012). It is also a good pointer towards the risk of CHF and one of the criteria that should be evaluated to know when to start treatment (Reynolds, et al., 2012; Keene, et al., 2019). Hence, it is clear that the assessment of the LA size is an essential part of the diagnostic evaluation for both patients suspected of having DMVD and those who are already diagnosed with it (Malcom, Visser, Philips, & Johnson, 2018). The gold standard is to obtain this measurement during an echocardiography, by indexing the LA to the aorta to serve as an internal control (Keene, et al., 2019).

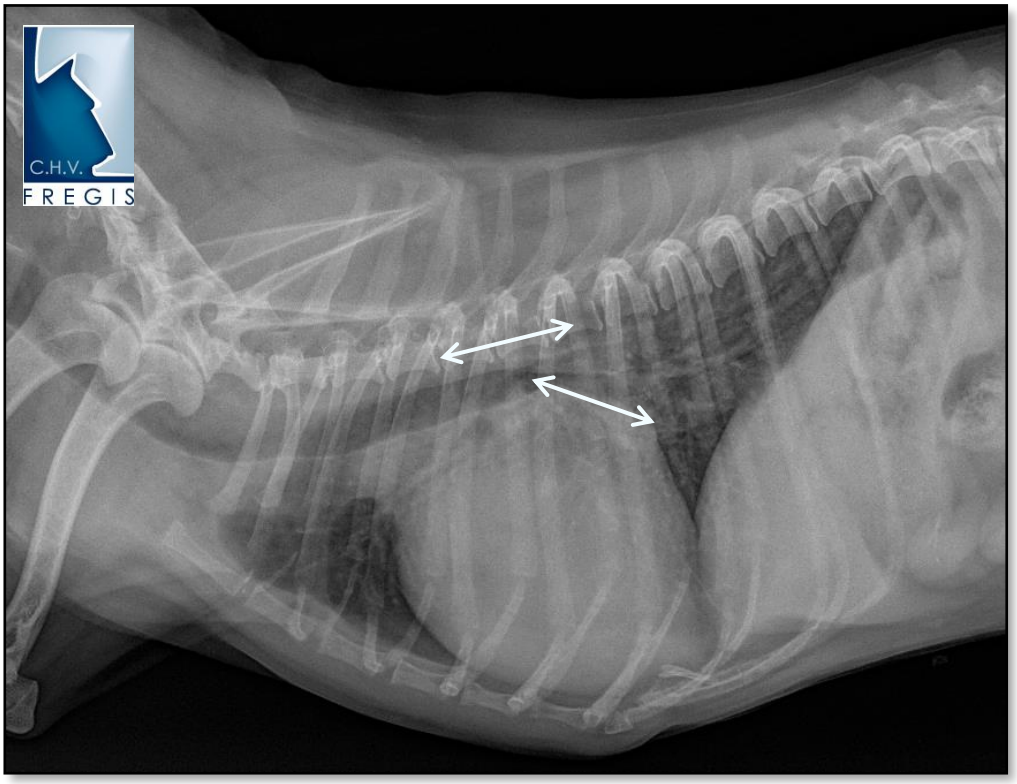
Owing to the fact that an echocardiographic assessment is not always an accessible option, efforts have been made to reach a quantitative method that accurately predicts LA enlargement radiographically. Nevertheless, doing so is primarily subjective and prone to error. One study in which a measurement of the tracheal bifurcation angle on DV radiographs of the thorax revealed that it was not a reliable way to estimate LA size (Le Roux, et al., 2012).

However, a recent study revealed a method, the VLAS, that is an accurate predictor of LA enlargement in a large and miscellaneous population of dogs with MMVD of varying degrees of severity. It was established that it is a repeatable method, with high level of agreement amongst measurements, whether it is in the same patient in different occasions or in multiple individuals. Moreover, it has a moderate positive correlation with echocardiographic measurements of LA size, and it is believed to be a valuable diagnostic tool when examining dogs with a left-sided apical systolic murmur concomitant with clinical signs like cough or respiratory difficulty (Malcolm, et al., 2018).

Since this is a more recently described method, digital calipers where used to determine the VLAS in lateral thoracic radiographs instead of the classic ones used in the VHS measurements. The first step is to draw a line that goes from the center of the most ventral aspect of the carina to the most caudal aspect of the LA at the site of intersection with the dorsal border of the caudal vena cava. The carina is the bifurcation of the left and right mainstem bronchus, identified as a radiolucent circular or ovoid structure within the trachea (Malcom, et al., 2018).

Afterwards, that line should be repositioned starting at the cranial edge of T4 and extending caudally ventral and parallel to the vertebral canal (see figure 15). The length of this line is then expressed in vertebral-body units to the nearest 0,1 vertebra, in the same manner as it is done in the VHS method (Malcom, et al., 2018).

Figure 15: Lateral radiographic projection of an 11-year-old dog demonstrating the VHS method. Original image, kindly provided by Dr. Eymeric Gomes from the Diagnostic Imaging Department of the Centre Hospitalier Vétérinaire Frégis.



Results of this study reveal that a VLAS ≥ 2,3 *vertebrae* is a radiographic indicator of LA enlargement. Additionally, dogs with a VLAS ≥ 2,3 *vertebrae* are expected to present a DMVD with important hemodynamic alterations (Malcom, et al., 2018).

Even though the VLAS has proved to be applicable to dogs with left-sided systolic murmurs and DMVD, further research is needed to determine the diagnostic value of this method in monitoring the progression of the disease and in assessing the LA size in patients with other cardiac diseases. Furthermore, similarly to the VHS, it would be more accurate to use breed-specific reference intervals, which requires the measurement of the VLAS in a large number of healthy dogs of various breeds (Malcom, et al., 2018)

4. Retrospective Study

4.1 Objectives

In view of the fact that DMVD has the highest prevalence of all canine heart diseases, but a medical cure is not available in the present days, it is of extreme importance to have tools that allow the monitoring of the disease (Das & Tashjian, 1965; Egenvall, et al., 2006; Gordon, et al., 2017). Since DMVD is a progressive disease, the necessity of adapting the treatment in accordance with the stage of the disease exists. Even though the best way to assess and definitely diagnose DMVD is by echocardiography, this exam requires additional expertise to be performed and interpreted, as well as substantial financial costs to the owner. Simultaneously, radiography of the thorax is widely available and cost-effective (Keene, et al., 2019). It is known that the evaluation of the size and shape of the cardiac silhouette on radiographs can provide useful information to proper diagnose and assess the severity of the cardiac disease (Buchanan, 2000).

There are two cardiothoracic ratios of main significance in radiographically evaluating patients with DMVD, namely the VHS and the VLAS (Buchanan, 2000; Malcolm, et al., 2018). The principal uses of the VHS method are to assess the presence of cardiomegaly in borderline cases, along with being able to do a quantification of the progression of heart enlargement over time in a given case (Buchanan, 2000). As far as the VLAS method is concerned, it has proved to be applicable to dogs with left-sided systolic murmurs and DMVD, but further research is needed to determine its diagnostic value in monitoring the progression of the disease (Malcom, et al., 2018).

In light of this information, a retrospective study was conducted in order to contribute to the comprehension of the uses of the VLAS method. In the study where the method was first described it was concluded that it is an accurate predictor of LA enlargement (Malcom, et al., 2018). However, by comparing the VLAS values of dogs with DMVD before and after the resolution of pulmonary edema with both echocardiographic measures and VHS values, an improved understanding of whether it permits to monitor the progression of the disease is expected to be reached. Moreover, knowing if and how the resolution of the pulmonary edema influences the heart measures is also one of the objectives.

To sum it up, the objectives of the present retrospective study were: 1) to monitor the progression of the LA and the LV size before and after the resolution of pulmonary edema using both the VLAS and the VHS methods; 2) to confirm that the VLAS method is an accurate predictor of LA enlargement in a sample of dogs with CHF; and finally 3) to assess if the LA size measures obtained by the VLAS method are correlated to those obtained echocardiographically.

4.2 Material and Methods

4.2.1 Animals

Case records of dogs, with DMVD and pulmonary edema, examined at CHV-Frégis by either Dr. Cecile Damoiseaux or Dr. Jean-Philippe Corlouer were reviewed retrospectively. All of the data was gathered and filtered from CHV-Frégis medical records under the supervision of Dr. Cecile Damoiseaux. The patients' information acquired from the clinical reports included: age, gender, breed, weight at the time of the occurrence of pulmonary edema, treatment plan and doses, thoracic radiographies, echocardiographic images and measurements, state of integrity of *chordae tendineae* and grade of heart murmur.

4.2.1.1. Enrolment criteria

Inclusion criteria

To be eligible for this study the dogs must have been given an echocardiographic confirmation of DMVD and present concomitant cardiogenic pulmonary edema. The diagnosis of pulmonary edema was done by clinical examination and thoracic radiography, and the animal must have been subsequently cured of the congestive signs. A second set of thoracic radiographs proving the absence of pulmonary edema must have been undertaken for each patient.

When first presented in consultation the animals had both an echocardiographic exam and a set of thoracic radiographs taken. DMVD was diagnosed based on the echocardiographic findings and was defined as the presence of irregularity, thickening and prolapse of the mitral valve apparatus, as well as evidence of mitral regurgitation during systole determined by color Doppler ultrasonography. Decompensated left-sided CHF was identified by virtue of radiographic signs of pulmonary edema that was considered cardiogenic in origin by a cardiologist and a specialist in diagnostic imaging, in addition to the existence of respiratory difficulty that revealed responsive to a treatment with furosemide. Absence of pulmonary edema was later confirmed radiographically by the same board-certified specialist in diagnostic imaging.

Exclusion criteria

Dogs were excluded from the study if any of the thoracic radiographs showed obvious malpositioning of the animal, incapacitating the achievement of the heart measurements. Patients that never fully recovered of the decompensated left-sided CHF who, consequently, never presented a second set of thoracic radiographs revealing the absence of pulmonary edema, were also not included. Proof of other pulmonary pathologies and/or clinically relevant cardiac disease (congenital or acquired) other than mitral regurgitation secondary to DMVD, was a criterion of exclusion as well.

4.2.2 Echocardiographic and Doppler Examination

Echocardiographic and Doppler examinations were carried out in standing awake animals with continuous ECG monitoring. The examinations were performed during the occurrence of pulmonary

edema by the Frégis' cardiology team mentioned above, in the day in which the animals were first presented at consultation (D0).

In order to evaluate the LA size, a standard right parasternal short-axis 2-D view, where the LA body, the Ao and the auricle are visible, was used to determine the ratio LA/Ao. This measurement was performed at the end of the diastole. Ventricular measurements were taken both in systole and in diastole from the right parasternal view using 2-D-guided M-mode according to the recommendations of the American Society of Echocardiography (Sahn, DeMaria, Kisslo, & Weyman, 1978). As far as the mitral regurgitation is concerned, it was calculated using spectral and color-flow Doppler in the left apical four-chamber 2-D view, aligning the regurgitant flow with the ultrasound beam. The presence or absence of *chordae tendineae* rupture was observed in the right parasternal long-axis 2-D view and the left apical four-chamber 2-D view, in a plane parallel to the long axis of the left-side heart chambers.

4.2.3 Radiographic Examinations

Right lateral and dorsoventral projections were used to evaluate the thorax of each dog before and after the treatment of pulmonary edema caused by DMVD. The images were used to confirm the presence of cardiomegaly and pulmonary edema, to exclude coexisting disease at inclusion into the study, to measure cardiac dimensions and, thereafter to confirm the absence of pulmonary edema.

The day in which the animals were first presented at consultation (D0), all of them presented pulmonary edema confirmed radiographically. The VHS measurements (VHS D0) of these radiographs, as well as the VLAS measurements (VLAS D0) were posteriorly measured with a digital caliper by a board-certified specialist in diagnostic imaging, using the methodology previously described in this dissertation.

A second set of radiographs was taken after the treatment of the pulmonary edema (D1) confirming its absence. Once again, the measurements of the VHS (VHS D1) and the VLAS (VLAS D1) were posteriorly done. The same clinician did the measurements before and after the treatment but was not present during the echocardiographic examinations, being blinded to the physical and echocardiographic examinations. Cardiomegaly was assessed with the VHS method and presence or absence of pulmonary edema was noted. For each dog, echocardiographic measurements were obtained before radiographic measurements. In order to better understand the variation of the VHS and VLAS measurements before and after the treatment of the pulmonary edema, the differences between the measurements from different days of the same animal were posteriorly calculated.

4.2.4 Statistical Analysis

Statistical Analysis were performed using Microsoft Office Excel 2016 and the statistical program Eviews version 9.

Data was expressed as mean values with respective range. For all analyses, a p value of <0.10 was considered significant, however, different significance values were considered, namely 1% (p value <0.01), 5% (p value < 0.05) and 10% (p value <0.10).

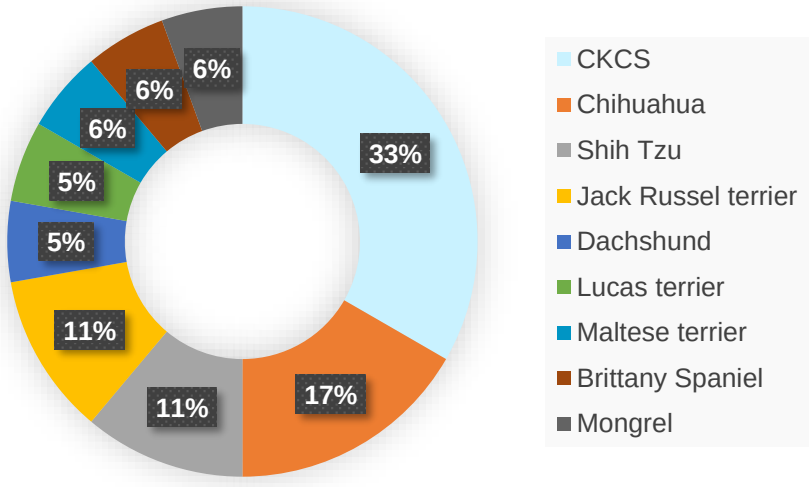
For the purpose of better understanding the relationship between some variables, 2 multiple linear regression models were formulated and the variable coefficients were estimated by the Robust Ordinary Least Squares Method. The normality of the distribution of each variable was tested with the Jarque-Bera Test. Moreover, in order to compare values of the same variable in different days, the Wilcoxon Test was used.

Concerning the descriptive statistics, the correlation matrix and the multiple linear regression models, they were obtained with the Eviews 9 software. As for the graphics, they were elaborated in the Microsoft Office Excel 2016.

4.3 Results

A total of eighteen cases were included in this study. All of the animals were classified as ACVIM Stage C patients, and all presented a left heart murmur (LHM), 72% had a grade IV in VI LHM and the remaining 28% presented a grade V in VI LHM. The mean age at presentation was 10 years (range, 7 – 16 years). The gender distribution was 11 males (61%), comprising 2 castrated and 9 intact, and 7 females (39%), 3 spayed and 4 intact. Multiples breeds were identified, namely CKCS (6/18), Chihuahua (3/18), Shih Tzu (2/18), Jack Russel terrier (2/18), Dachshund (1/18), Lucas terrier (1/18), Maltese terrier (1/18), Brittany Spaniel (1/18), and Mongrel (1/18). The chart 1 illustrates the prevalence of the different breeds. As for the mean weight at presentation it was 7.7 kg (range, 2.4 – 19.8 kg).

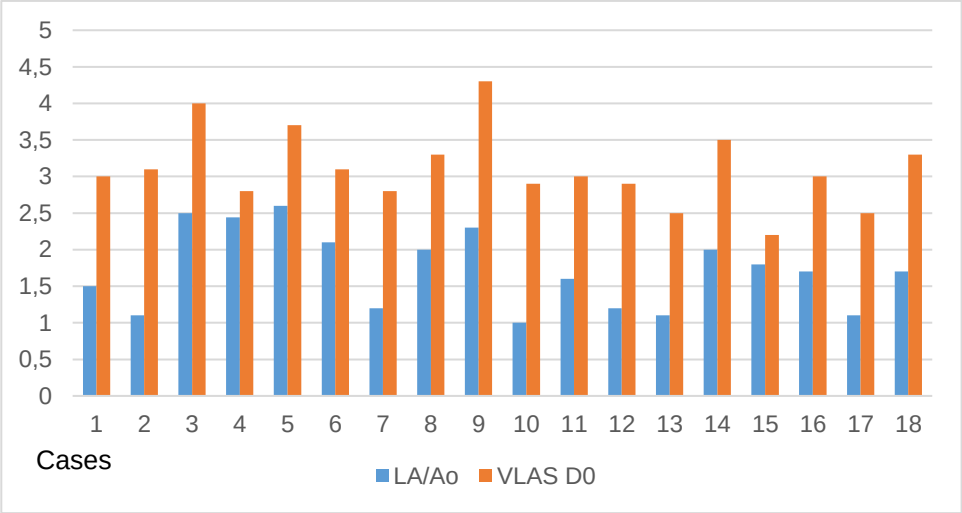
Chart 1: Prevalence of dog breeds in the group under study.



In the radiographs taken in the day in which the animals were first presented at consultation, the VHS D0 mean value was 12.55 *vertebrae* (range, 10.9 – 15.4 *vertebrae*) whereas the VLAS D0 mean value was 3.11 *vertebrae* (range, 2.2 – 4.3 *vertebrae*).

The echocardiographic exam performed in the same day revealed that concerning the dimension of the LA, the LA/Ao mean value was 1.72 (range, 1 – 2.6). The chart 2 illustrates the relation between the LA/Ao and the VLAS D0 measurements. The size of the LV was also examined, both in diastole and in systole. During diastole, the LV was dilated in 76% of the cases and normal-sized in 24%, as for during systole it was dilated in 53% of the animals and normal-sized in 47%. Only two animals had a normal-sized LV both in diastole and in systole. In regard to the integrity of the *chordae tendineae*, all dogs presented at least rupture of one of them. Amongst the group of 18 dogs, 11 had a principal *chordae* from the anterior leaflet ruptured, 4 presented not only a principal *chordae* from the anterior leaflet ruptured but also an accessory in the posterior leaflet, 1 had a principal *chordae* ruptured both in the anterior leaflet and the posterior leaflet, 1 had 2 principal *chordae* ruptured in the anterior leaflet, and 1 presented a rupture of an accessory *chordae* in the anterior leaflet. Another parameter observed was the regurgitation fraction, and its mean value was 65% (range, 45 – 87 %). Amid the dogs examined 5 out of 18 had a regurgitation fraction superior to 70%.

Chart 2: Column chart of VLAS D0 measurements versus LA/Ao measurements in D0 for the 18 dogs.



As a result of the diagnosis of pulmonary edema, a treatment with furosemide was initiated. The mean dose of furosemide (T) administered was 3.06 mg/kg/day (range, 0.93 – 5.70 mg/kg/day).
Concerning the number of days (ND) between the detection of the pulmonary edema and the day in which a second set of radiographs was taken confirming its absence (D1), its mean value was 17 days (range 6 – 66 days). Once again, the measurements of the VHS (VHS D1) and the VLAS (VLAS D1) were done revealing a VHS D1 mean value *vertebrae* of 12.19 (range, 9.8 – 14.7 *vertebrae*), and a VLAS D1 mean value of 2.92 *vertebrae* (range, 2.1 – 4.3 *vertebrae*).
In order to understand the variation of the VHS and VLAS measurements between the D0 and the D1, the difference of the values (D1-D0) was calculated. The mean value of the difference between VHS D1 and D0 measurements (DVHS) was -0.355 *vertebrae* (range, -1.7 – 0.6 *vertebrae*) and the difference between VLAS measurements (DVLAS) was -0.183 *vertebrae* (range, -1.1 – 0.3

vertebrae). These differences are graphically represented in the charts 3 and 4, respectively. Moreover, it was confirmed a significant difference between the VHS measurements from D0 and D1 (p value 0.05) as well as the VLAS measurements (p value 0.10).

Chart 3: VHS values of each animal before and after the treatment of pulmonary edema.

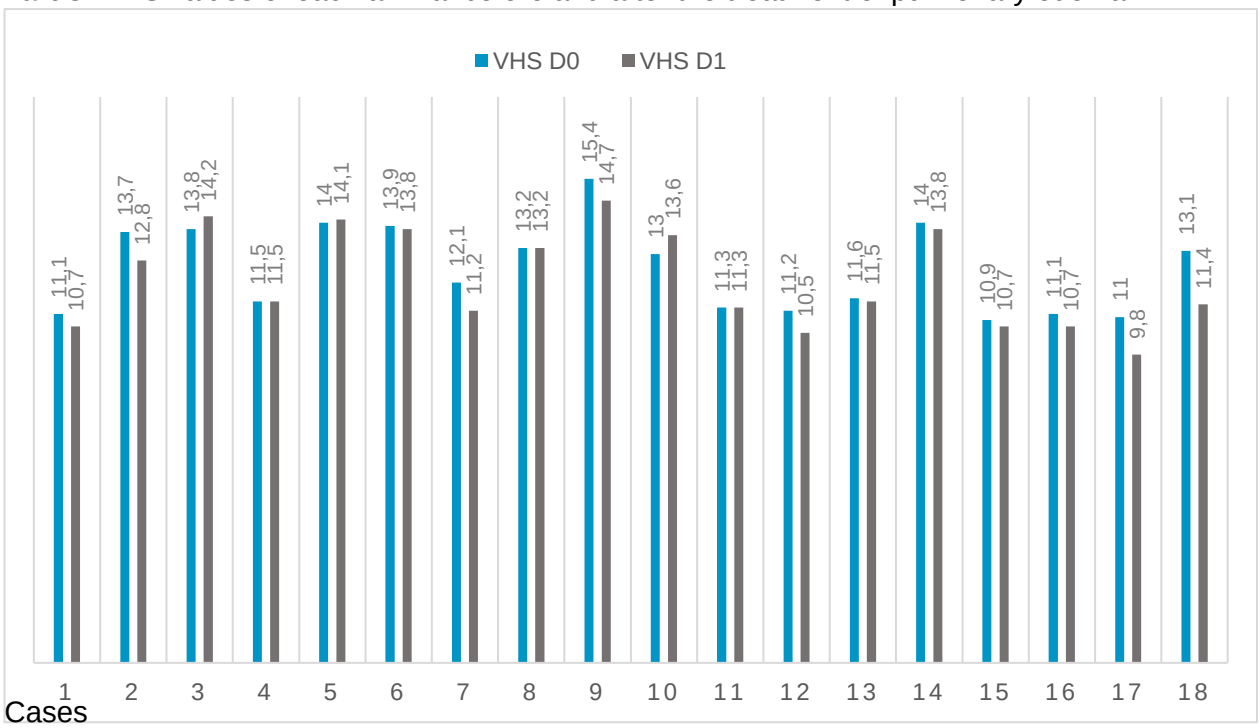
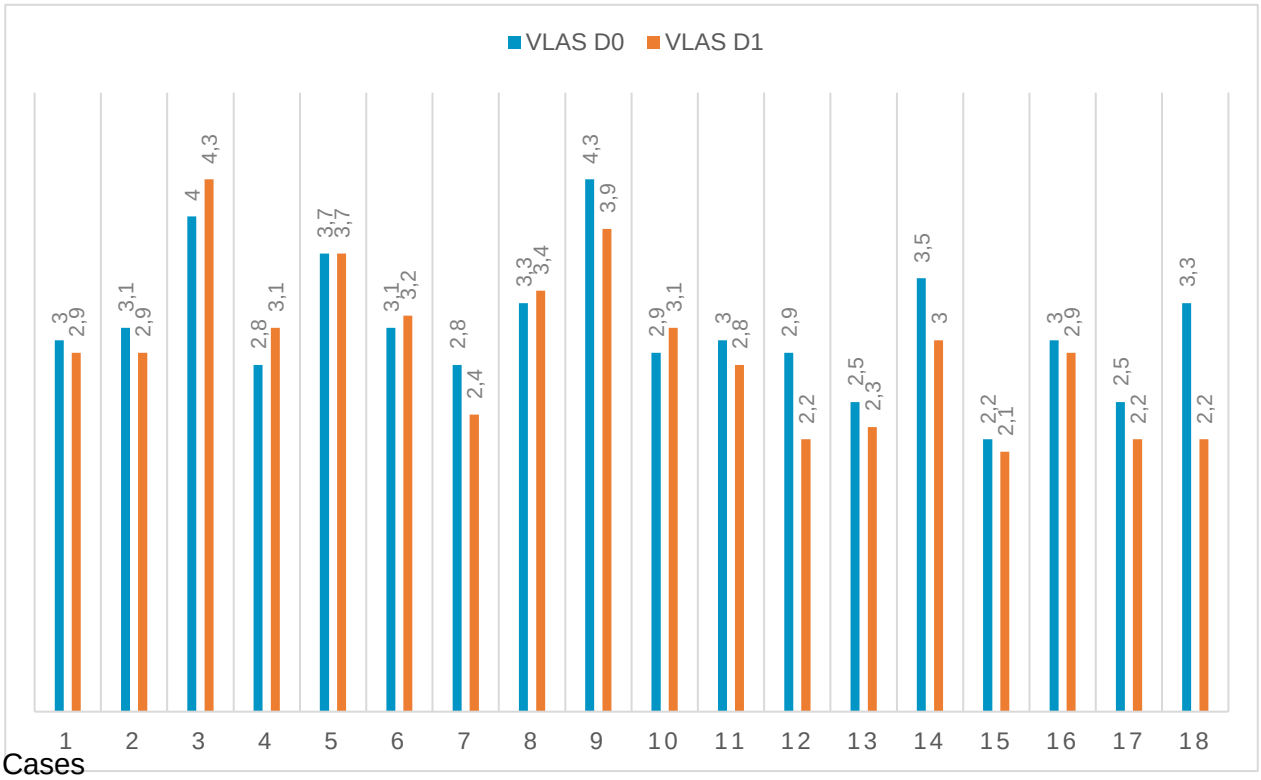


Chart 4: VLAS values of each animal before and after the treatment of pulmonary edema.



To assess the normality of the distribution of the variables, the Jarque-Bera test was done and it was determined that the variables ND and VLAS D0 do not follow a normal distribution. The descriptive statistics of a majority of the variables are schematized in the table 4, panel A.

Table 4: Descriptive Statistics and Correlation Matrix.

Legend: This table shows the descriptive statistics and the correlation matrix of the variables: Age (A), Dose of furosemide in mg/kg/d (T), Number of days between the presence and the absence of pulmonary edema

Panel A: Descriptive Statistics

	A	T	ND	LHM	VLAS D0	LA/Ao	DVHS	DVLAS
Mean	10.194	3.062	17.667	4.278	3.111	1.7189	-0.367	-0.206
Median	10.500	3.150	14.000	4.000	3.000	1.700	-0.200	-0.150
Max	16.000	5.700	66.000	5.000	4.300	2.600	0.600	0.300
Min	7.000	0.930	6.000	4.000	2.200	1.000	-1.700	-1.100
Std. Dev.	4.205	1.410	14.848	0.461	0.785	0.532	0.566	0.381
Jarque-Bera	2.485	1.025	32.455***	3.727	20.998***	1.290	1.032	1.687
p-value	0.288	0.599	0.000	0.155	0.000	0.525	0.597	0.430
Nº Obs.	18	18	18	18	18	18	18	18

Panel B: Correlation Matrix

	A	T	ND	LHM	VLASD0	LA/Ao	DVHS	DVLAS
A	1.000							
T	-0.328	1.000						
ND	-0.175	-0.035	1.000					
LHM	-0.045	0.275	-0.089	1.000				
VLAS D0	0.328	0.084	0.415	0.280	1.000			
LV/Ao	-0.621	0.421	0.500	0.073	0.025	1.000		
DVHS	-0.202	-0.014	-0.039	-0.038	-0.311	0.311	1.000	
DVLAS	-0.302	-0.221	-0.009	-0.158	-0.340	0.369	0.815	1.000

(ND), Left Heart Murmur (LHM), VLAS in the day when the first set of thoracic radiographs was taken, during the occurrence of pulmonary edema (D0) (VLASD0), Ratio between the diameter of the Left Atrium and the Aortic root (LA/Ao), Difference between the VHS measurement between the day when the second set of thoracic radiographs was taken, during the absence of pulmonary edema (D1) and D0 (DVHS), and Difference between the VLAS measurement between D1 and D0 (DVLAS). In the Panel A we can see the Descriptive Statistics information and in the Panel B the matrix correlations between the variables. Std. Dev. stands for Standard Deviation and Nº Obs. for number of observations. ***, ** and * represents significance value of 1%, 5% and 10% respectively.

A correlation matrix was elaborated and can be checked in the table 4, panel B. From the interpretation of this matrix, it can be verified that, on the one hand the variables A and DVHS, A and DVLAS, A and LA/Ao, A and LHM, A and ND, A and T, DVHS and ND, DVHS and VLAS D0, DVLAS and ND, DVLAS and VLAS D0, LHM and DVHS, LHM and DVLAS, LHM and ND, T and DVHS, T and DVLAS, T and ND are negatively correlated. On the other hand, the variables A and VLAS D0, DVHS and DVLAS, DVHS and LA/Ao, DVLAS and LA/Ao, LA/Ao and VLAS D0, LHM and LA/Ao, LHM and VLAS D0, ND and LA/Ao, ND and VLAS D0, T and LA/Ao, T and LHM, T and VLAS D0 are positively correlated.

As for the strength of the correlation, it is very strong between the variables DVHS and DVLAS (0.815); strong between the variables A and LA/Ao (-0.621); moderate between the variables T and LA/Ao (0.421), ND and LA/Ao (0.5000), ND and VLAS D0 (0.415); weak between the variables A and T (-0.328), A and DVHS (-0.202), A and DVLAS (-0.302), A and VLAS D0 (0.328), T and LHM (0.275), T and DVLAS (-0.221), DVHS and LA/Ao (0.311), DVHS and VLAS D0 (-0.312), DVLAS and LA/Ao (0.369), DVLAS and VLAS D0 (-0.340); very weak between the variables A and LHM (-0.045), A and ND (-0.175), T and DVHS (-0.014), T and ND (-0.035), T and VLAS D0 (0.084), LHM and DVHS (-0.038), LHM and DVLAS (-0.158), LHM and ND (-0.089), LHM and LA/Ao (0.073), LHM and VLAS D0 (0.028), DVHS and ND (-0.039), DVLAS and ND (-0.010), LA/Ao and VLAS D0 (0.025).

For the purpose of better understanding the relationship between some variables 2 multiple linear regression models were formulated. The multifactorial model (1) analyses if the DVLAS depends on the variables DVHS, ND, T and A.

$$DVLAS = \alpha_1 DVHS + \alpha_2 ND + \alpha_3 T + \alpha_4 A + \varepsilon_i \quad (1)$$

The multifactorial model (2) analyses if the DVHS depends on the variables DVLAS, ND, T and A.

$$DVHS = \alpha_1 DVLAS + \alpha_2 ND + \alpha_3 T + \alpha_4 A + \varepsilon_i \quad (2)$$

The α stands for the variable's coefficients. The results of the estimation of the coefficients of the models (1) and (2) can be seen in the table number 5.

Table 5: Estimation of the coefficients of the models (1) and (2).

Variable	Model (1) Coefficient	Model (2) Coefficient
DVHS	0.529***	-
	(0.000)	-
DVLAS	-	1.205***
	-	(0.000)
ND	0.002	-0.003
	(0.578)	(0.589)
T	-0.019	0.007
	(0.538)	(0.887)
A	-0.001	-0.004
	(0.907)	(0.790)
R^2	0.598	0.505

Legend: ***, ** and * represents significance value of 1%, 5% and 10% respectively. The values between ( ) are the *p values*. DVHS stands for the difference between the VHS measurement between the day when the second set of thoracic radiographs was taken, during the absence of pulmonary edema (D1) and D0, DVLAS for the difference between the VLAS measurement between D1 and D0, ND for the Number of days between the presence and the absence of pulmonary edema, T for the Dose of furosemide (mg/kg/d) and A for Age.

4.4 Discussion of Results

The main purpose of the study reported here was to compare the VLAS and the VHS measures in thoracic radiographs before and after the resolution of pulmonary edema in dogs with DMVD. As a result, all animals included in the study were ACVIM Stage C patients, which means that the whole group presented cardiac structural abnormalities with current clinical signs of heart failure, namely congestive signs such as pulmonary edema, and a LHM (Keene, et al., 2019). Overt pulmonary edema occurs when the capacity of the pulmonary lymphatic system is exceeded leading to an increase in the extravascular water content of the lungs (Murray, 2011). However, before this set of events takes place, a number of compensatory mechanisms are triggered with the objective of guarantying the forward stroke volume (Baumgartner, 2017).

The growing percentage of blood that flows backwards into the LA through the defective valve, creates the need of enlargement of the LA in order to keep an intra-atrial pressure lower than that of the LV, prompting the circulation of the blood flow in the right direction (Kittleson & Brown, 2003; Eriksson, et al., 2010). Therefore, it was expected that its size would be augmented in the study group, both echocardiographically and radiographically. This assumption was confirmed, since the VLAS D0 mean value of the animals studied was 3.11 *vertebrae* (range, 2.2 – 4.3), meaning that a majority of the animals studied presented a VLAS measurement ≥ 2,3 *vertebrae*, which is a radiographic indicator of LA enlargement (Malcom, et al., 2018). Furthermore, the ratio LA/Ao was also indicative of an enlarged LA, given the fact that the population's mean value was 1.72 in end-diastole (range, 1 – 2.6), which is compatible with stage ACVIM B (Keene, et al., 2019). In regard to those animals whose LA is not as enlarged, it can be explained by an abrupt raising of the pressure inside the atrium, giving it no time to properly adapt its size. Consequently, the sudden rise in pressure reflects backwards in the hydrostatic pressure of the pulmonary capillaries and is responsible for the development of pulmonary congestion and/or edema. Rupture of a principal *chordae tendineae* is an example of a situation that can lead to this succession of events (Ljungvall & Häggström, 2016). As previously mentioned in the results, all animals included in the studied group had at least one ruptured *chorda*.

In parallel with the main objective of this dissertation, it was also expected to achieve a confirmation of the accuracy of the VLAS method in predicting LA enlargement and its correlation with the echocardiographic LA/Ao measurement. Even though the strength of the correlation between these two variables is very weak (0.025), it is nonetheless positive, implying that when one augments the other does so too and vice-versa. The weak correlation is probably due to the fact that the number of individuals included in the study is small. Additionally, in the chart 2, it can be visually confirmed that a higher VLAS value corresponds to a higher LA/Ao value in the majority of cases.

While the LA enlarges to accommodate the regurgitant volume, the LV has to compensate for the loss of forward stroke volume and deal with the high end-diastolic pressures and volumes. To do so, in response to the volume overload, the ventricle wall suffers an eccentric hypertrophy (Grossman

& Paulus, 2013). The rate of increase in the LV dimensions is greater in the last year before the onset of CHF (Lord, et al., 2010). This explains why 16 of the 18 animals presented an enlarged LV in the echocardiographic exam performed during the occurrence of pulmonary edema. The other 2 cases may be accounted for by the lack of breed-specific normal reference ranges, which hampers the truthful judgement of the LV's size (Ljungvall & Häggström, 2016). Or by the sudden rise in pressure due to an acute rupture of a principal *chordae tendineae* (Ljungvall & Häggström, 2016).

Having in mind the determination of the heart's enlargement as a whole, the VHS measurement was performed. The different thoracic conformation of the various breeds makes it more accurate to use breed-specific normal ranges when analyzing this parameter (Lamb, et al., 2001). Nonetheless, in Buchanan and Bücheler's 1995 study it was determined that the mean VHS on lateral radiographs for 100 normal dogs of varying breeds was 9.7 +/- 0.5 *vertebrae*. In the present study, the VHS D0 mean value was 12.55 *vertebrae* (range, 10.9 – 15.4 *vertebrae*), indicating an enlarged heart. All of the individuals' VHS were compared to the breed-specific values showed in the table 3 when a value for the breed was available. After this comparation it was still evident that all the measurements were above the reference ranges, which is indicative of cardiomegaly.

The size of the heart was once again determined radiographically after the resolution of the pulmonary edema in order to evaluate the differences in size of the heart. Even though the VHS D1 mean value is lower (p value = 0.05) than that of D0 (12.19 *vertebrae versus* 12.55 *vertebrae*) it is still higher than the reference values, therefore the heart is still enlarged after the pulmonary edema (Buchanan & Bücheler, 1995). As far as the VLAS D1 measurements are concerned, its mean value proved to be lower (p value = 0.10) than that of D0 as well (2.92 *vertebrae versus* 3.11 *vertebrae*), but also still revealing an enlarged LA (Malcom, et al., 2018).

In order to understand the variation of the VHS and VLAS measurements between the D0 and the D1, the difference of the values (D1-D0) was calculated. The mean value of the difference between VHS D1 and D0 measurements (DVHS) was -0.355 *vertebrae* (range, -1.7 – 0.6 *vertebrae*) and the difference between VLAS measurements (DVLAS) was -0.183 *vertebrae* (range, -1.1 – 0.3 *vertebrae*). The negative value demonstrates that the measures from D0 are bigger than those of D1, for both VHS and VLAS measurements. Furthermore, the multiple linear regression models revealed that the variables DVHS and DVLAS are dependent on each other with a level of significance of 1%. As seen in the correlation matrix this correlation is positive and very strong (0.815) and also has a level of significance of 1%, implying that when one diminishes the other does so too and vice-versa. In other words, when the silhouette of the heart decreased in size, the LA decreased too.

The variation in the heart's size after the resolution of the pulmonary edema is probably linked to the diuretic effects of the furosemide. Furosemide is a potent loop diuretic, considered as one of the cornerstones in the management of acute and chronic pulmonary edema. It acts by reducing the intravascular fluid volume and therefore decreasing the preload venous and capillary pressures

(Chetboul, et al., 2017). In DMVD, the increase in the preload and reduction of the afterload, caused by the mitral regurgitation, triggers compensatory mechanisms, such as the enlargement of the heart chambers (O'Gara, et al., 2008). Once the furosemide reduces the venous and capillary pressures resolving the edema, the intra-cardiac pressures reduce as well, which may be the reason why there is a decrease in the VHS and VLAS measurements after the resolution of the pulmonary edema. Since the VHS only gives information on the overall size of the heart, we cannot infer on the effect of the diuretic treatment in the heart chambers separately. Nevertheless, it was demonstrated by the VLAS measurements that the LA decreases in size after the pulmonary edema, moreover, the difference in the LA's size is strongly correlated with the difference in the total heart's silhouette size. This can be due to the fact that what mainly suffers an alteration in size is the LA, but echocardiographic information on the heart chambers' size before and after the pulmonary edema is needed to confirm the statement.

The multiple linear regression models also revealed that neither the DVLAS nor the DVHS are significatively dependent on the age of the patient, the dose of the treatment with furosemide or the number of days between the presence and absence of pulmonary edema.

Another parameter observed was the regurgitation fraction, and its mean value was 65% (range, 45 – 87 %). Mitral insufficiency is normally considered as moderate when the regurgitation fraction values are higher than 30-50% and severe if higher than 70% (Kittleson & Brown, 2003; Gouni, et al., 2007). Amongst the dogs examined 5 out of 18 presented a severe mitral insufficiency and the other 13 a moderate one.

The recommended treatment for the cardiogenic pulmonary edema is 4 mg/kg of furosemide *per* day (Keene, et al., 2019). The mean dose of furosemide administered to the animals included was 3.06 (range, 0.93 – 5.70 mg/kg/d) which is a little lower than the ACVIM recommendations. However, it is always justifiable to try to use the minimum dose with efficacy (Keene, et al., 2019). Moreover, some dogs received torsemide, another loop diuretic, in association with furosemide. The dose of furosemide in these dogs was therefore decreased.

Concerning the number of days between the detection of the pulmonary edema and the day in which a second set of radiographs was taken confirming its absence, its mean value was 17 days (range 6 – 66 days) which means that the average time that took the animals to recover from the pulmonary edema was approximately 2 weeks. The correlation between the dose of furosemide and the number of days that the patients needed to recover is very weak and negative, meaning that the greater the dose fewer days would take the recovery and vice-versa. However, this correlation is not strong enough to draw suppositions.

In regard to the characteristics of the sample in study, they are in accordance with what is reported in other studies. The disease is age related and its prevalence increases drastically in 4 to 5-year old dogs. The mean age at presentation of the dogs studied is 10 years (range, 7 – 16), which follows the tendency mentioned (Whitney, 1974). Concerning the sex, DMVD is reported to be more

common in males than in females, and in the present study there are 61% of males. As for the breeds, DMVD can be encountered in all breeds but the highest prevalence is found in small to medium-sized dog breeds. The mean weight at presentation was 7.7 kg, which is a medium-sized dog weight. Furthermore, 33% of the dogs were CKCS and there were also Dachshunds and Chihuahuas which are among the breeds that more commonly develop the disease (Borgarelli & Buchanan, 2012).

Even though, DMVD has the highest prevalence of all canine heart diseases, animals in asymptomatic stages tend to stay stable and not progress into heart failure when properly treated and monitored (Ljungvall & Häggström, 2016; Borgarelli & Buchanan, 2012). This, allied with the rigorous inclusion criteria, accounts for the small size of the sample studied, since all animals had to be in symptomatic stages.

Limitations

The main limitations of this study are related to fact that it was done retrospectively. As a consequence, it was difficult to find clinical cases that reunited all of the information needed. Numerous dogs were excluded from the study because the owners did not want the realization of a thoracic radiography during the pulmonary edema or after, others because they rejected the echocardiographic examination, and others because they never brought the animal back to a control. As far as the author's opinion is concerned, this is probably due to lack of financial means to cover all the expenses of the multiple diagnostic exams. As a result, the sample of dogs included is not as large as desired.

4.5 Conclusion

The theoretical revision and retrospective study of these 18 clinical cases of DMVD allowed not only a deeper understanding of the disease physiopathology and management, but also permitted to acknowledge the complexity of its evolutive nature.

Given the high prevalence of dogs with DMVD and the absence of a cure in the present days, it is of extreme importance to have tools that allow the monitoring of the disease. Even though the best way to do so is echocardiographically, most small clinics do not have access to this diagnostic exam. Furthermore, some owners do not have the financial capacity necessary to proper follow the evolution of the disease with such an expensive exam. Simultaneously, radiography of the thorax is widely available and cost-effective, which justifies the importance of studying the evolution of the radiographic measures VHS and VLAS in dogs with DMVD.

Concerning the attempt to confirm the accuracy of the VLAS method in predicting LA enlargement and its correlation with the echocardiographic LA/Ao measurement, the size of the sample under study did not allow the drawing of a statistically significant conclusion. Nonetheless, it was verified that they have a positive correlation (0.025) with one another, implying that when one augments the other does so too, and vice-versa.

The main conclusion of this study is that the size of the LA and the cardiac silhouette decreases after the resolution of cardiogenic pulmonary edema when compared to the dimensions during its occurrence. Furthermore, this decrease in the LA's size is detectable using the VLAS method, which confirms its value in monitoring the progression of the disease. Consequently, it is possible for those who do not have access to an echocardiographic exam performed by someone with the required expertise, to use the VLAS method to follow the evolution of the LA's size throughout the progression of DMVD.

Further research is necessary to best correlate the radiographic measures with echocardiographic ones, as well as study a greater number of individuals.

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