PhD THESIS

The morphological validation of M-mode echocardiographic parameters of the left ventricle in adult dogs

(SUMMARY OF THE PhD THESIS)

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INTRODUCTION

Cardiac ultrasound, or echocardiography, is the "gold standard" method that allows non-invasive and non-ionizing visualization of the heart's internal structure, specifically the atrial and ventricular cavities, the atrioventricular orifices and valves, as well as the major vessels (pulmonary artery and aorta). Transthoracic echocardiography can be used in 2D, 3D, M-mode, and tissue mode. The dynamics of heart contractions are depicted through two-dimensional (2D) images and motion mode (M-mode), while blood flow can be visualized and measured using Doppler ultrasound.

Transthoracic echocardiography is considered the dominant cardiac imaging technique, which, due to its portability and versatility, is used even in emergency centre, operating rooms, and intensive care units. It is estimated that approximately 10% of canine patients seeking veterinary consultations suffer from primary heart disease, and among these, 75-80% are diagnosed with mixomatos mitral valve disease (MMVD) in various stages (KEENE ET AL., 2019). Recognizing the importance and frequency of canine heart disease, it is essential to establish a definitive diagnosis as well as the stage of disease progression, so the patient can receive appropriate therapy. The popularity and availability of echocardiography in veterinary practice, particularly for companion animals, have increased substantially in recent years.

M-mode and 2D mode are most commonly used in cardiac examinations of adult dogs. Before the implementation of M-mode, it was preceded by A-mode (amplitude mode) and B-mode (brightness mode). The addition of a time axis to B-mode led to the development of motion mode (M-mode) technique (CARERJ ET AL., 2003). Thus, M-mode echocardiography is derived from a line superimposed on a cross-sectional image, where all tissues intersected by this line are represented by a multitude of points with different echogenicity. In this way, the density and position of all tissues interposed in the path of a narrow ultrasound beam are displayed in a one-dimensional plane, where the long axis represents time. The unfolding of the image in M-mode produces an updated, continuously changing time sequence of the tissue section studied over several seconds. It is called M-mode because it illustrates a display of motion over a specific time interval (timed motion) (BOON, 1998; MERTENS ET AL., 2010). The control of speed and the repeatability of the technique allow not only excellent temporal resolution of moving structures but also precise measurements of wall thickness and cavity size (MERTENS ET AL., 2010). M-mode recordings have excellent temporal resolution and can complement 2D images by distinguishing certain structures such as trabeculae adjacent to the posterior wall, false tendons on the left side of the septum, or moderator bands on the right side of the septum (LANG ET AL.,

2005). Echocardiographic examination includes both qualitative and quantitative assessments, with M-mode being the most widely used quantitative method. Common M-mode measurements at the end of systole and diastole include left ventricular chamber diameter, left ventricular free wall thickness, interventricular septum thickness, and calculation of the shortening fraction (GUGJOO ET AL., 2014).

Establishing widely applicable reference intervals poses a challenge for canine patients, given the wide range of body sizes and somatotypes encountered in clinical practice. Common approaches to overcome this challenge include using breed-specific references, normalizing cardiac chamber measurements to another cardiac element such as the aorta, or normalizing ventricular cavity measurements to body weight using linear regression or an allometric scale. Although breed-specific reference intervals can offer advantages compared to normalization to body weight, they are not practical for all breeds and do not apply to mixed-breed dogs (VISSER ET AL., 2019).
In veterinary medicine, there is a limited availability of morphogeometric studies of a normal heart or a heart that has undergone a certain degree of remodeling. This aspect becomes increasingly relevant considering that ventricular geometry acts as an indicator of cardiac function. Therefore, anatomical studies aimed at obtaining morphogeometric data of the left ventricle in healthy dogs are necessary to establish reference values for canine cardiac morphogeometric analysis (CARDOSO ET AL., 2023). Moreover, although veterinary medicine is continuously advancing, the anatomical study of cardiac cavity geometry is in its early stages, despite the contribution it can offer to the development and improvement of therapeutic protocols, surgical techniques, cardiac medical devices, or pharmacological therapies (CARDOSO ET AL., 2023).

The **aim** of the research was to anatomically validate the left ventricular parameters in adult dogs, obtained through M-mode echocardiography.
In this context, several objectives were established for the research:

- Establishing inclusion criteria for the biological material in the research to form a representative group of adult dogs without cardiac pathologies;
- Performing M-mode echocardiography to create a database of left ventricular parameter measurements in the selected group of dogs;
- Conducting necropsies and morphometric examinations of the heart in the studied dogs;
- Comparing the left ventricular parameters obtained in M-mode with the measurements obtained during necropsy;
- Comparing the obtained morphometric measurements with those in the specialized literature;

- Determining the heart weight/body weight ratio and left ventricular weight/body weight ratio, and subsequently comparing the obtained data with those published in the literature to date;
- Measuring troponin I, a biomarker specific to cardiac remodeling;
- Interpreting the histological samples collected from the dogs included in the study to exclude the presence of cellular changes;
- Determining the cardiac pH to establish the phase of cardiac resolution, specifically the optimal time frame for performing morphometric measurements.

THE STRUCTURE OF THE PHD THESIS

The thesis titled "*Morphological validation of left ventricular echocardiographic parameters in M-mode in adult dogs*" comprises 132 pages, written according to current academic and national editing standards. The doctoral thesis is structured into two parts, consisting of 9 chapters and includes 35 figures, 40 tables, and 206 bibliographic references.

The first part of the thesis, titled "The Current State of Knowledge," is structured into 3 chapters on 23 pages. In the first chapter (**Chapter 1**), information is synthesized regarding the introduction and development of M-mode in veterinary echocardiography, specifically: the characteristics and measurements determined in M-mode in dogs, the importance of M-mode in mitral valve disease in dogs, the standardization of M-mode, M-mode depending on breed, the current needs of M-mode, and also echocardiography in M-mode in other species. Additionally, the most relevant cardiac biomarker, troponin I, is presented (**Chapter 2**). The last chapter of this part (**Chapter 3**) includes the methodology for a complete cardiac necropsy examination, with an emphasis on canine cardiac morphometry. This chapter also includes histological examination.

The second part of the doctoral thesis is dedicated to original research and is titled "Personal Contribution." It is structured into 6 chapters on 75 pages.

Chapter 4 presents the research aim and objectives. The aim of the research was to morphologically validate the left ventricular values obtained echocardiographically through M-mode in adult dogs. To achieve this aim, 9 objectives, mentioned earlier, were pursued.

Chapter 5 represents an introduction to the research topic.

Chapter 6 of the research synthesizes the materials and methods used. This study was conducted on a population of adult dogs from Cluj and the surrounding county. The selection of patients was based on a set of criteria classified as general, echocardiographic, and morphological. The initial group comprised 45 dogs, of which 17 were excluded. The final group consisted of 28 adult dogs, represented by 16 males and 12 females, with an average age of 9.7 years and an average weight of 25.3 kg. Of the total 28 adult dogs, 11 were mixed breeds, 3 were German Shepherds, 2 were Labrador Retrievers, 2 were Dachshunds, and there was one representative each of the breed: Tosa Innu, Rottweiler, Cocker Spaniel, Golden Retriever, Bichon Frise, German Shorthaired Pointer, Dogo Argentino, Irish Setter, West Highland White Terrier, and Boxer. All patients followed a standard protocol that included history taking, recording physiological constants, followed by a complete cardiological examination with a focus on the right parasternal window, short axis view at the level of the papillary muscles, where left ventricular parameters were measured at the end of systole and diastole using M-mode, followed by troponin I measurement, patient euthanasia, necropsy, and morphometric examination of the heart. Finally, the statistical tests used to explore the relationship between morphological and echocardiographic determinations were described.

Chapter 7 is dedicated to the results and discussions. This chapter addresses the individual results and corresponding discussions for each stage as follows: the results and discussions of the clinical examination; the results and discussions of the electrocardiographic (ECG) examination; the results and discussions of troponin I measurement; the results and discussions of echocardiographic measurements; the results of the necropsy and morphometric examinations, along with discussions on the morphometric results; the results and discussions of cardiac ratios; the results and discussions of myocardial pH; and the results and discussions of the histological examination. The chapter concludes with the results and discussions concerning the achievement of a morphometric validation of the left ventricular parameters determined in M-mode.

In **Chapter 8,** the conclusions and recommendations based on the obtained results are presented. **Chapter 9** includes the originality and innovative contributions of the thesis. The originality and innovative contributions of this thesis lie in its structure and the working methods used, which make a significant contribution to the field of veterinary cardiology. The major innovative contribution of this thesis is the research objective itself: to compare morphological values with echocardiographic values, an aspect that has not been explored in the specialized literature, particularly on this specific topic in adult dogs.

RESULTS OF THE OWN RESEARCH

The most relevant results obtained from the anamnesis and clinical examination highlighted a geriatric population dominated by a combination of comorbidities, with the most frequent being neoplastic, renal, and neurological pathologies. Additionally, a statistically significant association ($p<0.05$) was found between age and cardiac pathology, as well as between heart murmur and cardiac pathology. The electrocardiography results were dominated by the presence of a considerable number of patients who exhibited premature ventricular extrasystole as the only arrhythmic alteration. Troponin I was measured in only 21 patients, with the remaining samples being excluded due to hemolysis. A small number of patients presented normal serum values. The results of troponin I measurement indicated elevated serum levels in cases where cardiac pathology was associated with non-cardiac pathology.

The echocardiographic results were systematically organized in tables for each individual included in the study. Of the 28 patients, 15 exhibited minor echocardiographic alterations. The most frequently affected valve was the mitral valve, with this alteration being associated or not with other valvulopathies (in 14 of the 15 cases). Tricuspid valve involvement, along with other valvulopathies, was present in 6 cases, aortic valve involvement in 5 cases, and pulmonary valvulopathy was diagnosed in 4 patients. No patient exhibited a singular valvulopathy of the tricuspid or pulmonary valve. One patient presented with aortic insufficiency without other valvulopathies. Three patients exhibited only mitral regurgitation, classified as MMVD stage B1 according to ACVIM. The most common concurrent valvulopathies included mitral and tricuspid regurgitation in 6 patients, followed by triple valvulopathy in 3 patients involving the mitral, aortic, and pulmonary valves. One patient exhibited ulcerative vegetative endocarditis, with dual involvement of the mitral and aortic valves.

In mixed-breed patients, compared to the values estimated by BOON (2011), increased values were observed for IVSd, LVPWd, and LVPWs, and lower values for LVIDd, IVSs, and LVIDs. Comparing the left ventricular parameters in M-mode by breed also showed differences, although much less pronounced than those observed in mixed breeds. However, a similar variation trend was maintained, with an overall increase in IVSd values and a decrease in LVIDd and LVIDs values compared to the standard. There were 2 patients whose values fell within the reference range for all parameters.

During the necropsy examination, all valvular insufficiencies determined by echocardiography were validated. The morphometric determinations showed small differences compared to the study published by QUEIROZ ET AL. (2018), although only the thickness of the left ventricular free wall could be compared. Macroscopically, two myocardial changes were identified: a neoplasm and an ischemic lesion. These were described histopathologically.

In the context of cardiac ratios, the group's average values fell within the reference ranges. The most significant ratio proved to be the heart weight/body weight ratio, which was also the most studied: 0.43%-0.99% (ROBINSON AND ROBINSON, 2015), 0.6% -1.1% (QUEIROZ ET AL., 2018), 0.61%-0.94% (BIENVENU AND DROLET, 1991), and 0.66%-1.20% (CARVALHO ET AL., 2002). A wider range was reported by GHOSHAL (1986), with a heart weight/body weight ratio of 0.5% to 2.2% (QUEIROZ ET AL., 2018). SCHONING ET AL. (SCHONING ET AL., 1995) published similar data for Greyhounds, with results of 1.3 ± 0.2% for females and 1.2 ± 0.2% for males. Contrary to the literature, which suggests that the value of the ratio is higher in males (ROBINSON AND ROBINSON, 2015), or that sex does not influence this ratio (QUEIROZ ET AL., 2018), in the present study, the percentage of the ratio was higher in females.
The results of myocardial pH determinations were recorded as they were, without reference values for comparison. The values obtained could serve as a basis for future similar studies and might become an optional element in post-mortem cardiac examinations regarding the quality of the heart and the examination of appropriate myocardial tissue. The average pH was 5.89, with a maximum value of 6.93 and a minimum of 4.77, the latter being the only pH value below 5.

Histopathologically, the valvular changes of mitral endocardiosis and ulcerative-vegetative endocarditis were validated. All muscle tissues collected from the areas where the measurements were taken showed a normal structure. A few incidental changes observed included arteriosclerosis, represented by a compact, hyaline material at the level of the intima or media of the blood vessels, as well as the presence of an adipocyte infiltration at the myocardial level. The macroscopic neoplasm was confirmed to be a hemangiosarcoma, and the second macroscopic change observed was described as a cardiac thrombus in formation, with myocardial fibrosis and mononuclear infiltration.

The results of the statistical tests were organized into tables. Based on the statistical results, we can conclude that systolic measurements tend to estimate the gold standard values better than diastolic ones.

GENERAL CONCLUSIONS

Chapter 8 of the thesis presents the general conclusions and recommendations of the research:

1. **Age, weight, and breed** are the most important factors that can influence M-mode echocardiographic values in adult dogs. Furthermore, age is also a risk factor for the presence of cardiac pathology, serving as a significant indicator for the decision of euthanasia.
2. **Troponin I** is a cardiac biomarker with indicative value, capable of showing elevated serum levels in both cardiac and non-cardiac conditions. A cardiac pathology that progresses simultaneously with another condition leads to a considerable increase in the serum level of Troponin I in dogs. The use of a

human Troponin I assay kit has significant limitations regarding the results and interpretation of serum values in a clinical context for adult dogs.

3. **Heart murmurs** are a good predictor of the presence of cardiac pathology in dogs.
4. **Post-mortem myocardial pH determination** can be a good indicator of an optimal heart for macroscopic evaluation. At 12-24 hours post-mortem, the normal cardiac pH value averages 5.89 (±0.43).
5. **Cardiac morphometric values and the heart weight/body weight ratio** are essential for conducting a complete necropsy examination of the heart.
6. The **heart weight/body weight ratio** is not influenced by stage B1 of mitral endocardiosis according to ACVIM classification.
7. **Echocardiographic values of left ventricular parameters** at end-systole determined by M-mode in an adult dog with a heart with normal morphology and function tend to estimate morphometric values better than those recorded at end-diastole.
8. **Canine echocardiography** is a useful and precise tool for evaluating a heart with normal function and structure, validated by macroscopic aspects, morphometric measurements, and histopathological examination.
9. The use of **breed-specific echocardiographic reference intervals** is a more appropriate approach for evaluating cardiac structure and function.

RECOMMANDATIONS

- Each institution or diagnostic center should establish reference intervals and normal heart values for healthy dogs within the local population. It has been proven that what is considered normal can vary in a complex manner depending on the region. The research results can serve as a valuable database regarding the echocardiographic and morphometric values of a normal heart in adult dogs from this geographical area.
- For future studies, it is recommended to assemble a group of patients that is representative of a specific population.
- The management of cardiac pathology is not influenced solely by M-mode measurements of the left ventricle. Therefore, future investigations should also focus on parameters of the right ventricle, the two atrial chambers, and the major blood vessels.

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