DOPPLER ECHOCARDIOGRAPHIC ASSESSMENT OF ACQUIRED
HEART DISEASES IN DOGS

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CHENNAI – 600 051
2014
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Thesis submitted in part fulfilment of therequirements for the degree of DOCTOR OF PHILOSOPHY in VETERINARY CLINICAL MEDICINE, ETHICS AND JURISPRUDENCE

to the
TAMIL NADU VETERINARY AND ANIMAL SCIENCES UNIVERSITY
CHENNAI

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CHENNAI – 600 051
2014
Dedication

To My Beloved Family
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CERTIFICATE

This is to certify that the thesis entitled "DOPPLER ECHOCARDIOGRAPHIC ASSESSMENT OF ACQUIRED HEART DISEASES IN DOGS" submitted in part fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY in VETERINARY CLINICAL MEDICINE, ETHICS AND JURISPRUDENCE to the Tamil Nadu Veterinary and Animal Sciences University, Chennai is a record of bonafide research work carried out by THIRUNAVUKKARASU, P. under my supervision and guidance and that no part of the thesis has been submitted for award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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ACKNOWLEDGEMENT

I express my deep sense of gratitude and heartfelt thanks to my Chairman Dr. B. Nagarajan, Professor and Chairman of Advisory Committee, Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Madras Veterinary College whose spirit and passion for science, ideas to deal with the research problems and involvement with his originality have exceptionally inspired me and enriched my growth as a student. His efforts and scientific support made this postgraduate a very learning experience and this thesis output as most valuable one.

I am very much thankful to members of advisory committee Dr. K. Kumanan, Director of Research, Tamil Nadu Veterinary and Animal Sciences University and Dr. S. Ramesh, Associate Professor and Head and, Centralised Instrumentation Laboratory for his suggestions and encouragement during this study.

I acknowledge with profound thanks to Dr. A. P. Nambi, Professor and Head and member of advisory committee, Dept. of Veterinary Clinical Medicine, Ethics and Jurisprudence for his valuable ideas, encouragement, suggestions and profitable discussions at every stage of this study.

I place on record my sincere thanks and gratitude to Dr. S. R. Srinivasan, Director of Clinics; and Dr. S. Prathaban, Dean, VCRI, Tirunelveli, Tamil Nadu Veterinary and Animal Sciences University for their guidance, valuable advice and constant encouragement throughout the study.

I acknowledge with thanks the valuable help from Dr. P. S. Thirunavukkarasu, Professor and Head, Department of Clinics, in my study period.

I place on record my sincere thanks and gratitude to Dr. K. Jeyaraja, Assistant Professor, Dept. of Clinics, for his guidance, valuable advice, constant encouragement, enormous support and technical skill throughout my study period.

My sincere thanks to Dr. Cecilia Joseph, Dr. K. Kulasekar, Dr. P. Sridevi, Dr. P. Devendran, Dr. M. Kathirvelan, Dr. T. Sivakumar, Dr. K. N. Selvakumar, Dr. M. Thirunavukkarasu, Dr. D. Kathiresan, Dr. G. Rathina Sabapathy, and Dr. S. Vairamuthu for their support in this thesis period.

I am sincerely thanking Dr. G. Suganya, Dept. of VPY; and Mrs. Savithri, Lab Asst, and Dr. K. Vijayarani, Dept of ABT, MVC for their immense help in ELISA studies.
My sincere thanks to Dr. S. Selvam & Dr. G. Kathiravan, Dept. of AHSCA, MVC without their support this could not be achieved.

I extend my sincere thanks to Dr. S. Kavitha and Dr. M. Chandrasekar, Associate Professors, and Dr. M. Balagangathara Thilagar, Assistant Professor of Dept. of Veterinary Clinical Medicine, Ethics and Jurisprudence; and Dr. B. Gowri, Dr. D. Chandrasekar, Dr. G. R. Baranidharan, Dr. C. S. Arunaman and Dr. C. Jayanthi, Assistant Professors, Dept. of Clinics, Madras Veterinary College; Dr. V. Vijayanand, PGRIAS; and Dr. P. Selvaraj, VUPH; Dr. R. V. Suresh, Professor (Retd.) for their constant encouragement and support throughout the period of study.

I express my sincere thanks to Dr. M. Thangapandiyan and Dr. C. Balachandran for their help in necropsy studies.

I express my warm gratitude and sincere thanks to Post-graduates Dr. P. A. Enbavelan, Dr. M. Sandya Bhavani, Dr. Snehlatha, Dr. R. C. Sundarrajan, Dr. Hamsha Yamini, Dr. A. Arun and Dr. A. Sivaprakasam for their kind support and timely help.

I express my sincere gratitude to Mrs. S. Indhumathi, Mrs. Parameswari, Mrs. G. S. Krishnaveni and Mrs. D. Rani, VCMEJ, for their immense help and cooperation during the study period.

I definitely thank my friend Dr. R. Ramesh for the timely help in my struggling period.

Before I conclude, I feel privileged to thank Dean, MVC; Registrar, TANUVAS and Vice-chancellor, TANUVAS and all University Officers for their support and help during this study period.

I express my deep sense of thanks and warm gratitude to my loving family members Mrs. R. Dhanalakshmi, Mrs. K. Indira and P. T. Hanisha; brother Mr. P. P. Arulkumar & family; sister Mrs. P. Suseela & family; and Mr. R. Krishnamoorthy & family and children P. Suriyaprasath & A. Nithra; my friends Dr. N. Ramaswami and Dr. S. Rajendran and without whose support I couldn’t have achieved this study.

(P. THIRUNAVUKKARASU)
Acquired heart diseases (AHD) are common and often fatal when it leads to CHF in dogs and it occurs most often secondary to degenerative mitral valve disease (MVD), dilated cardiomyopathy (DCM), pericardial diseases and Hypertrophic cardiomyopathy (HCM). Early recognition of AHD is of clinical importance. Tissue Doppler echocardiography is an ideal tool to evaluate patients with acquired heart diseases, offering the potential to improve early identification and management of AHDS.

The objectives of this study were to quantify the global and regional myocardial function by Tissue Doppler echocardiography in acquired heart diseases of dogs; to compare the diagnostic efficacy of different modes of Tissue Doppler echocardiography in dogs; and to establish the usefulness of biochemical marker in diagnosis of acquired heart diseases in dogs.

106 animals with acquired heart diseases were selected based on echocardiographic findings from the animals that were brought to MVC teaching hospital and they were grouped as apparently healthy dogs, DCM, MVD, Pericardial diseases and HCM groups.

Parameters of the study included Prevalence analysis, Medical history, Clinical presentation, Baseline Haematology panel, Baseline serum biochemistry panel, Radiography, Doppler BP, ECG, Echocardiographic indices such as 2-D...
echocardiographic indices, M-Mode Echocardiographic indices and Colour flow Doppler, Tissue Doppler Imaging and NT-proBNP Assay.

The incidence of AHDs was found to be 0.37 per cent in the five semester study period. Labradors and Spitz were found to be commonly affected with DCM and MVD respectively. Older male dogs were found to be more commonly affected. The observed chief complaints included inappetance, exercise intolerance, abdominal enlargement, syncope and weakness. Tachycardia, ascites and murmurs were the common clinical signs in all the groups of AHDs. Haematological assessment showed no significant changes. Serum biochemical assessment showed significant hypernatremia in all groups except HCM. Radiographic signs of AHDs included cardiomegaly, pulmonary oedema and left atrial enlargement. Dogs with DCM and pericardial effusion had significantly elevated VHS and confirmed the presence of acquired heart disease.

ECG findings in AHDs were the DCM dogs had characteristic arrhythmic pattern of atrial fibrillation in 20.69 per cent cases and atrial flutter in 8.62 per cent of cases. MVD group dogs had atrial enlargement in 51.28 per cent; pericardial effusion group had low voltage QRS complex and electrical alternans in 83.33 per cent; and HCM group had ventricular enlargement in 100.00 per cent cases.

Echocardiographic findings in DCM were LV dilatation, LA dilatation, increased LA/Ao ratio, decreased LVFW and septal thickness, decreased FS, increased LVIDd &LVIDs, increased EPSS and secondary regurgitation of mitral valve and Tricuspid valve; and in MVD were LA dilatation, increased LA/Ao ratio, FS<60 per cent with cardiac failure were identified and mild, moderate and severe MR was found in colour flow Doppler; in pericardial effusion were echo-free space around ventricular chambers, Right Atrial collapse indicating tamponade; and in HCM were LV hypertrophy, SAM and increased FS were observed.

In pulsed wave tissue Doppler highly significant decrease in Sm and Em/Am ratio all groups; and significant decrease in Em in all groups were observed; and significant increase in Am in pericardial effusion and HCM were observed. Very typical Em/Am ratio reversal (Em/AM<1) was observed in pericardial effusion and HCM group.

In NT-proBNP assay a highly significant increase in levels was observed in DCM, MVD with systolic failure, MVD without systolic failure and in occult cardiac diseases which indicated that this marker is highly sensitive and specific for cardiac diseases. Post-mortem examination of DCM dogs reveals the heart was enlarged, globular, and flabby in appearance
with rounded apex due to generalized ventricular dilatation on gross pathology and on transverse biventricular slice of heart the marked dilatation of lumen, attenuation of papillary muscles and thinning of free walls of ventricles were observed. On histopathology left ventricular myocardium showed attenuated wavy fibre type of change.

In this study the following conclusions made were Tissue Doppler Echocardiography of acquired heart diseases the regional and global velocities were highly significantly decreased in dogs; Pulsed wave Tissue Doppler Echocardiography is very effective in assessing the peak myocardial velocities in real time, when compare to 2D Tissue Doppler Echocardiography and Colour M-mode Tissue Doppler Echocardiography in dogs and the cardiac biomarker NT-proBNP was very effective and specific marker and very useful in diagnosing and categorizing the cardiac diseases in dogs.

*Keywords: Echocardiography, Tissue Doppler Imaging, NT-proBNP, Dogs*
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<td>Two Dimensional</td>
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<td>AF</td>
<td>Atrial Fibrillation</td>
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<td>AHD</td>
<td>Acquired Heart Diseases</td>
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<tr>
<td>ALP</td>
<td>Alkaline phosphatase</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine transaminase</td>
</tr>
<tr>
<td>Ao</td>
<td>Aorta</td>
</tr>
<tr>
<td>AS</td>
<td>Aortic Stenosis</td>
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<tr>
<td>AT</td>
<td>Atrial Tachycardia</td>
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<tr>
<td>AVJT</td>
<td>Atrioventricular Junctional Tachycardia</td>
</tr>
<tr>
<td>BP</td>
<td>Blood pressure</td>
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<tr>
<td>BUN</td>
<td>Blood Urea Nitrogen</td>
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<tr>
<td>BW</td>
<td>Body weight</td>
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<tr>
<td>CHF</td>
<td>Congestive Heart Failure</td>
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<tr>
<td>CT</td>
<td>chordae tendineae</td>
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<tr>
<td>CTR</td>
<td>chordae tendineae rupture</td>
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<tr>
<td>DCM</td>
<td>Dilated cardiomyopathy</td>
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<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
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<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EF%</td>
<td>ejection fraction</td>
</tr>
<tr>
<td>EPSS</td>
<td>E-Point to Septal Separation</td>
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<tr>
<td>ESVI</td>
<td>end-systolic volume index</td>
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<tr>
<td>FS%</td>
<td>fractional shortening</td>
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<tr>
<td>FT</td>
<td>Fractional Thickening</td>
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<td>HCM</td>
<td>Hypertrophic Cardiomyopathy</td>
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<td>IVCT</td>
<td>Isovolumic Contraction Time</td>
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<td>IVRT</td>
<td>Isovolumic Relaxation time</td>
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<td>IVS</td>
<td>Inter Ventricle Septum</td>
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<tr>
<td>LA</td>
<td>Left Atrium</td>
</tr>
<tr>
<td>LA/Ao</td>
<td>left atrium to aorta ratio</td>
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<td>LV</td>
<td>Left Ventricle</td>
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<td>Description</td>
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<td>LVFW</td>
<td>Left Ventricular Free Wall</td>
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<td>LVID</td>
<td>Left Ventricle in Diastole</td>
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<td>MR</td>
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<td>MV</td>
<td>Mitral Valve</td>
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<td>MVD</td>
<td>Mitral Valvular Disease</td>
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<tr>
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<td>NSR</td>
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<td>NT-proBNP</td>
<td>N-terminal pro-B-type natriuretic peptide</td>
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<td>PAH</td>
<td>pulmonary arterial hypertension</td>
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<td>PE</td>
<td>Pericardial Effusion</td>
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<tr>
<td>PW</td>
<td>Pulsed Wave</td>
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<tr>
<td>RA</td>
<td>Right Atrium</td>
</tr>
<tr>
<td>RF</td>
<td>regurgitant fraction</td>
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<tr>
<td>RV</td>
<td>Right Ventricle</td>
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<td>RVID</td>
<td>Right Ventricle in Diastole</td>
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<tr>
<td>SAM</td>
<td>Systolic Anterior Motion</td>
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<tr>
<td>SBP/DBP</td>
<td>Systolic Blood pressure/Diastolic Blood pressure</td>
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<td>TDI</td>
<td>Tissue Doppler Imaging</td>
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<td>TDE</td>
<td>Tissue Doppler Echocardiography</td>
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<td>TR</td>
<td>Tricuspid Regurgitation</td>
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<td>VHS</td>
<td>Vertebral Heart Score</td>
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CHAPTER I
INTRODUCTION

Small animal medical practice is being met with ever increasing challenges, very much given to the increasing population of pet animals, especially the geriatric populations and their age related diseases.

Congestive heart failure (CHF) is the inability of the heart to provide adequate circulation to meet the body’s needs. It is the end result of a weakened heart muscle. The health of the liver, kidneys, lungs, and other organs is impaired by the CHF, resulting in a problem involving multiple organs.

A diseased heart can compensate for many months or years without signs of failure (Calvert et al., 1996 and Koch et al., 1996). When failure does occur, it may appear suddenly and unexpectedly-sometimes immediately after strenuous exercise, when the heart is unable to keep up with the body’s demands.

Acquired heart diseases are the major silent killers in dogs, similar to human beings. Acquired heart diseases (AHD) are common and often fatal when it leads to CHF in dogs characterized by cardiac dysfunction, neuro-hormonal activation, sodium and water retention and increase in left ventricular (LV) filling pressures (LVFP). It occurs most often secondary to degenerative mitral valve disease (MVD), dilated cardiomyopathy (DCM) and pericardial diseases. Hypertrophic cardiomyopathy (HCM) is another AHD which is a rare form of heart muscle disease in dogs. It is characterized by a thickening of the walls of the heart. This extremely rare disease usually affects young male dogs. Most dogs with HCM will not exhibit any symptoms of the disease and the cause is unknown.

Early recognition of AHD is of clinical importance. AHD can be suspected by clinical signs although reliability of such findings may be limited. Unfortunately, common clinical signs of congestive heart failure (CHF), such as coughing and difficulty in breathing, are nonspecific and may mimic respiratory disease, making diagnosis difficult.
Diagnosing heart disease and/or heart failure in the dog requires a combination of several different testing methods. Thoracic radiography was the most commonly applied method for the diagnosis of AHD. However, radiography is of unspecified sensitivity and specificity, especially in the setting of combined heart and lung disease. An electrocardiogram is the only diagnostic test that can be used to detect and diagnose heart arrhythmias (abnormal heart beats or rhythms). Arrhythmias can occur with AHDs.

Doppler Echocardiography is a simple and quantifiable non-invasive method for estimation of volume status and filling pressures. It could not only refine the diagnosis, but also promote early recognition of AHD, advance optimal medical management and facilitate therapeutic monitoring. Doppler echocardiography (DE), because of its non-invasive nature, is an ideal tool to evaluate patients with acquired heart diseases, offering the potential to improve early identification and management of these patients.

More recent advances in ultrasound technology with the introduction of newer imaging modalities, such as Tissue Doppler Imaging (TDI), Strain (St) and Strain Rate (SR) imaging and 2-dimensional (2D) Speckle Tracking Echocardiography (STE) have provided new parameters to assess myocardial performance, including regional myocardial velocities and deformation, ventricular deformation, ventricular twist, and mechanical synchrony (Chetboul, 2010). Pulsed Wave Tissue Doppler Imaging (PW TDI) enables global and regional myocardial function to be quantified from measurements of myocardial velocities in real time (Chetboul, 2002). TDI has contributed to a better understanding of the nature of myocardial dysfunction that is associated with acquired heart diseases, thus providing new insights into their pathophysiology and suggesting the possibility of new therapeutic approaches. TDI is more useful not only in assessing systolic but also in diastolic myocardial functional impairment. TDI is more sensitive than conventional echocardiography in detecting pre-clinical myocardial abnormalities before the occurrence of left ventricular dilation and dysfunction (Chetboul, 2010).

The natriuretic peptides are produced in the myocardium as preprohormones, which are subsequently cleaved first into prohormones (e.g.
proBNP, proANP) and then mature into active hormones. The cardiac biomarker NT-proBNP is a 76 amino acid N-terminal fragment of brain natriuretic peptide which regulates fluid homeostasis. NT-proBNP level in the blood is used for screening, diagnosis of congestive heart failure (CHF) and may be useful to establish prognosis in heart failure (Oyama and Singletary, 2010). The plasma concentration of NT-proBNP is typically increased in patients with asymptomatic or symptomatic left ventricular dysfunction (Moonarmart, 2010; Oyama and Sisson, 2007). The patients with respiratory signs and exercise intolerance may be easily differentiated from cardiac patients by estimation of these peptides (Kellihan et al., 2011). This marker may be very useful for diagnosis of heart diseases even in the absence of echocardiography. With this backdrop the following objectives were taken for the study.

The objectives of this study included:

- To quantify the global and regional myocardial function by Tissue Doppler echocardiography in acquired heart diseases of dogs
- To compare the diagnostic efficacy of different modes of Tissue Doppler echocardiography in dogs
- To establish the usefulness of biochemical marker in diagnosis of acquired heart diseases in dogs
CHAPTER II
REVIEW OF LITERATURE

2.1 ACQUIRED HEART DISEASES

Acquired heart disease accounted for a vast majority of cases of heart disease in dogs in which the most common were myxomatous valve disease (MVD) and dilated cardiomyopathy (DCM) with variation in prevalence owing to local differences in breeds (Häggström et al., 1992).

2.1.1 Aetiology

Ortiz-Lopez et al. (1996) considered that autosomal dominant, autosomal recessive, X-linked and mitochondrial modes of inheritance were responsible for DCM. McEwan (1998) opined that Newfoundland was genetically predisposed to develop DCM.

Sisson et al. (1999) classified secondary cardiomyopathies according to their aetiology as drug- or toxin-induced, genetic, infiltrative, ischemic, metabolic, nutritional, or inflammatory. McEwan (1999) opined that familial DCM had been shown in a number of breeds and in most of these an autosomal dominant mode of inheritance was suspected.

Meurs et al. (2001) opined that Dilated Cardiomyopathy in dogs had many unique attributes for each affected breed, including age of onset, sex predisposition, rate of progression, presence and type of arrhythmias, response to nutritional supplements and tendency to die suddenly or develop congestive heart failure and also concluded that DCM might be an x-linked recessive trait in Great Danes.

Meurs et al. (2007) reported that DCM in the Doberman pinscher was a familial disease inherited as an autosomal dominant trait and the causative gene(s) responsible for this condition remained unresolved.

2.1.2 Acquired Valvular Heart Disease

2.1.2.1 Mitral Valve Disease (MVD)

Whitney (1974) described chronic valvular disease as the most common cardiac abnormality in dogs, which might be associated with valvular incompetence, systolic murmurs and congestive heart failure.
Kittleson et al. (1984) and Thomas (1987) opined that mitral regurgitation due to mitral valve fibrosis was a common cause of congestive heart failure in dogs.

Mitral valve prolapse was reported as an important component of devastating myxomatous mitral valve disease leading to congestive heart failure in small breeds of dogs (Häggström et al., 1994 and Pedersen and Olsen, 1998).

Chronic valvular disease was commonly observed in dogs of small to medium sized breeds with progressive thickening and contraction of atrioventricular valves, leading to insufficient coaptation of leaflets. Causing an increase in regurgitation of blood into ipsilateral atrium. Areas of contact along the free border of mitral cusp and chordae tendinae cordis were commonly affected but tricuspid valves also might develop pathologic changes. Pulmonary and aortic valves seldom were affected (Swenson et al., 1996).

Hyun (2005) opined that severe mitral regurgitation could cause left ventricular volume overload, which might predispose animals to cardiac arrhythmia and ultimately lead to left sided heart failure.

Mitral regurgitation due to myxomatous mitral valve diseases had been reported to account for 75 per cent of the cases of decompensated heart failure in dogs (Hansson et al., 2002; Häggström et al., 2004 and Smith et al., 2005).

Mitral valve prolapse might be primary or secondary. Primary mitral valve prolapse resulted from intrinsic abnormalities of the mitral valve leaflets, usually myxomatous degeneration and secondary mitral valve prolapse was present without inherent pathologic valvular abnormalities and was typically secondary to haemodynamic causes such as volume contraction and reduced left ventricular size or myocardial diseases resulting in akinetic muscle (Boon, 2006).

Valvular stenosis was recorded mostly always from congenital abnormality, and valvular regurgitation might develop from congenital malformation or acquired disease (Rush and Bonagura, 2006).

Chetboul and Tissier (2012) reported that worsening of MR leads to several combined complications including cardiac remodelling, increased left
ventricular filling pressure, pulmonary arterial hypertension, and myocardial dysfunction.

2.1.3 Myocardial Diseases

2.1.3.1 Dilated Cardiomyopathy (DCM)

Ettinger et al. (1970) defined idiopathic Dilated Cardiomyopathy (DCM) as congestive heart failure in conjunction with dilatation of the cardiac chambers and absence of other clinically important cardiovascular disease.

Darke (1985) reported primary (idiopathic) Cardiomyopathy in giant breed dogs in which loss of myocardial contractility was accompanied by severe dilation of cardiac chambers.

Sisson and Thomas (1995) were of the opinion that Dilated Cardiomyopathy (DCM), a myocardial disease, was an important cause of congestive heart failure and sudden death in dogs affecting primarily large and giant breeds and in Doberman pinscher. (Calvert et al., 1997 and Calvert et al., 1982).

DCM was found to be characterised by impaired myocardial contractility, elevated filling pressure and progressive dilatation of the left, right or all four chambers of the heart, without proportionate compensatory myocardial hypertrophy and the asymptomatic stages of the occult DCM, if undetected, might lead to sudden death or congestive heart failure (McEwan, 2000).

Dilated cardiomyopathy was reported as one of the most common acquired heart diseases, leading to high morbidity with poor prognosis and high mortality in dogs (Tidholm et al., 2001).

Tidholm and Jonsson (2005) opined that dilated cardiomyopathy could also be associated with hypothyroidism which was one of the major metabolic disorders related with the particular cardiac disorder in dogs.

2.1.3.2 Hypertrophic Cardiomyopathy (HCM)

HCM was characterized by unexplained concentric LV hypertrophy where the left ventricular chamber was not dilated and often smaller than normal (Liu,
In HCM diastolic failure secondary to impaired relaxation was a common complication resulting in elevated LV filling pressure and a dilated LA chamber (Kovacic and Muller, 2003).

Fox (2003) stated that hypertrophic cardiomyopathy was characterized by increased cardiac mass associated with a non-dilated, hypertrophied left ventricle. Phenotypic variability is substantial and includes both diffuse and segmental forms of left ventricular hypertrophy associated with dynamic obstruction to left and right ventricular outflow and diastolic dysfunction, and heart failure.

2.1.4 Acquired Pericardial Disorders

2.1.4.1 Pericardial Effusion

The reported causes in pericardial effusion in dogs include intrapericardial neoplasia, idiopathic haemorrhagic pericarditis and LA rupture secondary to chronic mitral regurgitation (Berg and Wingfield, 1984; Cobb and Brownlie, 1992; De Madron, 1991). Shaw and Rushb (2007) stated that the most common causes of pericardial effusion include cardiac hemangiosarcoma, idiopathic pericardial effusion, and chemodectoma and Shaw and Rusha in 2007 reported that most cases of pericardial effusion can be diagnosed with a thorough physical examination. Physical examination findings may include muffled heart sounds, pulsus paradoxus, and jugular venous distention. Radiographs may show a globoid cardiac silhouette and Echocardiography was reliable in diagnosing pericardial effusion.

2.2 HEART FAILURE

Ware et al. (1990) described degenerative mitral valve disease and idiopathic dilated cardiomyopathy as the two most common causes of spontaneous heart failure in dogs.

McDonagh et al. (1998) defined congestive heart failure as the end stage of progressive deterioration of left ventricular function, which could remain asymptomatic for many years.
Martin (2003) described that development of heart failure was not only due to the cardiac muscle injury but also exhibited when the compensatory hemodynamic and neurohormonal mechanisms became overloaded or exhausted.

Jortani et al. (2004) defined heart failure as a complex clinical syndrome manifested by signs and symptoms of low cardiac output and pulmonary or systemic congestion; and abnormalities in left ventricular function and neurohormonal regulation were major characteristics of this condition.

Identifying, classifying and grading heart failure were pre-requisites for selection of appropriate therapeutic intervention and required a robust, practical and affective definition of the condition (Steg et al., 2005).

Thomas et al. (2006) concluded that significant numbers of patients appeared to have heart failure with neither systolic dysfunction nor defined diastolic abnormalities. Based on modern echocardiographic criteria and further insisted to diagnose heart failure utilising a combination of symptoms and elevation of circulating B-type natriuretic peptide.

2.2.1 Pathophysiology of the Heart Failure

Congestive heart failure was associated with an increased renin-angiotensin-aldosterone system activity in dogs and human (Kluger et al., 1982 and Teerlink, 1996) and systemic activation of the renin-angiotensin-aldosterone system increases retention of sodium and water, leads to arteriolar vasoconstriction and elicited thirst (Lumbers, 1999).

Heart failure secondary to mitral regurgitation could be due, not only to myocardial failure, but also to severe regurgitation by itself or in combination with myocardial failure (Kittleson et al., 1984).

Packer et al. (1987) reported that congestive heart failure was accompanied by an elevation in circulating catecholamines, particularly norepinephrine.

Hirsch et al. (1990) opined that increased renin-angiotensin-aldosterone system activity might lead to modification of myocytes and myocardial architecture by inducing myocyte hypertrophy and necrosis at the local level (Tan
et al., 1991) and fibrosis, which was mediated through increased collagen synthesis which lead eventually to systolic dysfunction (Wilke et al., 1996).

Floras (1993) observed a decreased β-adrenergic responsiveness to endogenous or exogenous agonists. Koch et al. (1995) found that both plasma rennin activity and plasma aldosterone concentration were increased in symptomatic DCM dogs and plasma rennin activity was also increased in some DCM asymptomatic dogs too; while plasma aldosterone concentration was not.

Borgarelli et al. (1999) reported that dogs with symptomatic DCM and occult DCM had significantly lower density of total adrenoreceptors, β₁ and β₂ adrenoreceptor subtypes on their lymphocytes as compared to the control group.

Dzimiri (1999) observed that the down regulation of β-adrenoreceptors had been associated with an increased activity of α adrenoreceptors in DCM dogs.

Re et al. (1999) observed that both in lymphocyte and myocardial cell membranes, all subtypes (total, β₁, β₂ and α₁ adrenoreceptor) appeared to be down regulated in heart failure.

Re et al. (1999) and Borgarelli et al. (1999) observed that in symptomatic DCM dogs, plasma catecholamine concentration was significantly higher. Unger (2000) opined that the augmented levels of plasma catecholamines; in particular the release of norepinephrine by adrenergic cardiac nerves increased both myocardial contractility and heart rate resulted in increased cardiac workload and myocardial cell death.

McEwan (2000) opined that dilated cardiomyopathy involved elevated filling pressure and eventually resulted in the onset of clinical signs associated with left, right or biventricular congestive heart failure.

In heart failure, a reduction in cardiac output led to activation of the sympathetic system producing peripheral vasoconstriction, with positive inotropic and chronotropic effects on the heart, aiming to restore cardiac output and blood pressure towards normal. Prolonged activation of these mechanisms might cause myocardial cell death, peripheral vasoconstriction, myocardial hypertrophy and fibrosis inducing tachycardia and arrhythmia (Martin, 2003).

2.2.2 Diastolic Heart Failure
Schober and Fuentas (2002) stated that diastolic dysfunction is a common event in boxer dogs with advanced Aortic Stenosis. Severity of Aortic Stenosis, LV hypertrophy, and diastolic abnormalities such as delayed relaxation or increased myocardial stiffness are related with each other and may be diagnosed using echocardiographic indices. A pseudonormal Doppler mitral inflow pattern may be anticipated in these dogs and should not be confused with a normal filling pattern. Systolic function is normal in most dogs with mild or moderate AS. Left ventricular systolic dysfunction is a rare finding, however, if present it is a sign of advanced disease and poor prognosis in Aortic Stenosis. Long-axis shortening of the LV should be considered when assessing LV systolic function.

### 2.2.3 Classification of Heart Failure

Heart failure might result from inability of the heart to eject blood properly (systolic failure) or from inadequate filling (diastolic failure) or both. Heart failure could also be classified based on the side of failing as right, left or bilateral failure (De-Morais and Schwartz, 2005).

#### 2.2.3.1 International Small Animal Cardiac Health Council System (Miller and Tilley, 1995)

- **Class I**: asymptomatic.
  - **Class IA**: no evidence of compensation for underlying heart disease. (No volume overload or pressure overload detected radiographically or echocardiographically.)
  - **Class IB**: clinical signs of compensation for underlying heart disease. (Volume overload or pressure overload detected radiographically or echocardiographically.)
- **Class II**: mild to moderate heart failure with clinical signs at rest or with mild exercise. Treatment required.
- **Class III**: advanced heart failure; clinical signs of severe congestive heart failure.
  - **Class IIIA**: home treatment possible.
  - **Class IIIB**: requires hospitalization.

#### 2.2.3.2 The modified NYHA functional classification of heart failure
The modified NYHA functional classification of heart failure can be summarized as follows:

- **Class I** describes patients with asymptomatic heart disease (eg, CVHD is present, but no clinical signs are evident even with exercise).
- **Class II** describes patients with heart disease that causes clinical signs only during strenuous exercise.
- **Class III** describes patients with heart disease that causes clinical signs with routine daily activities or mild exercise.
- **Class IV** describes patients with heart disease that causes severe clinical signs even at rest.

### 2.2.3.3 ACVIM Consensus Statement (Atkins et al., 2009)

- **Stage A**: patient at risk of developing heart disease in the future, eg patient from breed with high predisposition for cardiac disease.
- **Stage B**: asymptomatic patients with evidence of structural heart disease, eg presence of murmur:
  - **B1**: with no evidence of cardiac remodelling (radiographically or echocardiographically).
  - **B2**: with evidence of cardiac remodelling.
- **Stage C**: patients with clinical signs of congestive heart failure (either past or present).
- **Stage D**: refractory heart failure. Patients showing clinical signs in spite of standard treatment for congestive heart failure.

### 2.3 DEMOGRAPHY

#### 2.3.1 Prevalence

Congestive heart failure (CHF) was reported as one among the most frequently encountered cardiac diagnoses, with an estimated prevalence of one per cent (Fisher et al., 2001). Häggström et al. (2004) documented that heart failure had been accounted for 7.3 per cent of mortality in dogs in an age of below 10 yrs. and the particular condition was listed as the third most common cause of death in dogs of that age group.
Myxomatous degeneration was not restricted to the mitral valve and it might be detected in any of the four intracardiac valves. The incidence of valve involvement in dog was reported as follows: 62 per cent incidence of mitral valve alone, 32.5 per cent incidence of mitral and tricuspid valves and 1.3 per cent incidence of tricuspid valve alone (Buchanan, 1977). The pulmonary and aortic valves are less commonly affected (Häggström et al., 2004).

Vollmar (1999 and 2000) diagnosed Dilated Cardiomyopathy in 66 (16.5 per cent) dogs out of 400 Irish wolfhounds and in 121 (24.2 per cent) dogs in a clinical study of 500 Irish wolfhounds.

O’Grady and O’Sullivan (2004) reported that the occurrence of DCM was 0.3 per cent in Labrador retrievers, (73 out of 21,501 Labrador retrievers) as referred from the University of Purdue Veterinary Medical Database 1985 to 1991. Martin et al. (2010) reported DCM in 20 Labrador retrievers out of 367 clinical cases.

### 2.3.2 Breed

A high incidence of chronic congestive heart failure in male English Cocker Spaniels was reported by Detweiler (1964).

The prevalence of valvular incompetence leading to heart failure was higher in certain small and medium sized breeds including Poodle, Schnauzer, Chihuahua, Doberman Pinscher, Fox Terrier, Boston Terrier and Cocker Spaniel (Das and Tashjian, 1965; Detweiler and Patterson, 1965).

Poodles, Schnauzers, Chihuahuas, Fox terriers, Boston terriers and Doberman pinschers were more commonly affected than other breeds and German shepherd was infrequently affected (Buchanan, 1977).


Gooding et al. (1982) recognized cardiomyopathy in 23 Cocker Spaniels from a single kennel. Thrusfield et al. (1985) noticed a strong predisposition to heart valve incompetence in the Cavalier King Charles Spaniel.

Thomas (1987) diagnosed eight young Cocker Spaniels with severe congestive heart failure due to congestive cardiomyopathy.
Mitral valve prolapse might be seen without any evidence of insufficiency and seems to be genetically predisposed in Cavalier King Charles Spaniels (CKCS) and Dachshunds. These dogs showed prolapse as early as 3 years of age without any clinical signs of murmurs and had high incidence of mitral valve insufficiency later in life (Beardow and Buchanan, 1993). Breed predisposition for subaortic stenosis had been recognized in Rottweiler, Boxer, German short haired pointer and Samoyed (Kienle et al., 1994 and Ware, 2003).

Right ventricular cardiomyopathy was documented in a male Dachshund with severe congestive heart failure (Simpson et al., 1994).

Sisson and Thomas (1995) reported that the most commonly affected breeds of DCM as Scottish deerhound, Doberman pinscher, Irish wolf hounds, Great Danes, Boxers, Saint Bernard, Afghan hound and Newfoundland.

Tidholm and Jonsson (1996) in a study of 189 dogs of 38 different breeds reported that no major breed specific differences concerning clinical, pathological or prognostic characteristics were found. The authors were of opinion that Newfoundland breed as one of the most commonly affected breed with DCM (Tidholm and Jonsson 1997; Menaut et al., 2005; Martin et al., 2009).

Dilated cardiomyopathy had been commonly reported in middle to old age group in large and giant breed dogs such as Doberman Pinschers, Boxers and Great Danes (Tidholm et al., 1997), whereas clinical signs of congestive heart failure resulting from mitral valve endocardiosis were observed almost exclusively in geriatric small breed dogs (Abbott, 1998).

Dambach et al. (1999) reported that canine Dilated Cardiomyopathy was breed-specific, which implied that certain clinical and pathological characteristics of DCM were specific for certain breeds, such as for Doberman Pinschers, Boxers, English Cocker Spaniels, and lately also for Portuguese Water Dogs.

Vollmar (1999) suggested a hereditary component to dilated cardiomyopathy in certain breeds like Doberman pinscher, Deer hound and Irish wolfhound. McEwan (2000) documented that Dobermans had a particularly malignant form of DCM, which was faster and progressive in nature. This finding
was further supported by Meurs et al. (2007), who described DCM as a familial disease inherited by an autosomal dominant trait in this breed.

Petric et al. (2002) in a study of 52 dogs of different breeds with DCM, reported 21 (39 per cent) in Doberman pinchers and 31 (61 per cent) in other breeds.

O’Grady and O’Sullivan (2004) stated that DCM occurred more frequently in large and giant breed dogs like Doberman pinscher, Irish wolfhounds, Saint Bernard dogs, German boxers, Great Danes, German shepherds, Newfoundland and Bull-mastiff, whereas a relatively high prevalence has been reported in medium sized dogs like English and American cocker spaniels and Dalmatians (McEwan et al., 2003; Koch et al., 1993).

Martin et al. (2009) in a study of DCM in 369 clinical cases of 35 different breeds observed in which 20 Labrador retrievers were affected with DCM. He also reported that in England, DCM occurred primarily in dogs of medium to large sized pure bred dogs with Dobermans and boxers being the most common.

High incidence of pericardial effusion in large breed dogs with the highest incidence seen in golden retrievers, German shepherd, Saint Bernard, Labrador retrievers, and Newfoundlands (Johnson et al., 2004; Aronsohn and Carpenter, 1999; Berg et al., 1984; Gibbs et al., 1982)

2.3.3 Age

Detweiler et al. (1968) noted that the prevalence of the disease increased with advancing age so that approximately 10 per cent of 5 to 8 year old dogs, 20 to 25 per cent of 9 to 12 year old dogs and 30 to 35 per cent of dogs over 13 years age exhibited murmurs.

Sisson and Thomas (1995) reported that, age at onset of clinical signs varied considerably, although most dogs were initially presented at the age of five to seven years. Even puppies could be affected within first week or months after birth, as described in Portuguese Water Dogs (Dambach et al., 1999) and more recently in a litter of Dobermans (Vollmar et al., 2003)

*Myocardial Diseases*
Tidholm and Jonsson (1996) in a study with DCM reported that the onset of clinical signs ranged from 3.5 months to 11.7 years, with a mean of five years. Tidholm et al. (1997) in a study with DCM observed that the age at onset of clinical signs ranged from 3.5 months to 13 years with a mean of 6.6 years.

Prevalence of DCM in Doberman pinscher was found to be increased with age (Calvert et al., 1997 and Meurs, 1998).

Vollmar (2000) reported the mean age of Irish wolf hound with DCM as 4.2± 2.1 years. Meurs et al. (2001) in a study of Great Dane with DCM observed that the age at the time of diagnosis was 1.5 to 8 years with mean of 4.8±2.3 years. Petric et al. (2002) reported the average age of Doberman pinchers affected with DCM as 6.5±1.9 years. Martin et al. (2010) in a study of DCM observed the mean age at the time of diagnosis as 78 months with a range of 56 to 102 months.

Meurs et al. (2007) reported an adult onset (median of 7.5 years) of dilated cardiomyopathy in Doberman Pinschers.

Wess et al. (2010) observed the prevalence of DCM in Doberman pinschers in various age groups and reported that 3.3 per cent between 1 to < 2 years of age, 9.9 per cent between 2 to 4 years of age, 12.5 per cent between 4 to 6 years of age, 43.6 per cent between 6 to 8 years of age, 44.6 per cent in the age group of 8 years. and the cumulative prevalence of Doberman pinchers cardiomyopathy as 58.2 per cent.

Valvular Diseases

Thrusfield et al. (1985) reported that there was high prevalence of chronic valvular disease in young age.

Häggström et al. (2000) diagnosed myxomatous valve disease in 103 Cavalier King Charles Spaniel with an average age of 7.5 years. Mitral regurgitation and mitral valve diseases were recorded in 50 small breeds of dogs with an average age and body weight of nine year and sixteen kg. respectively (Thomason et al., 2007).

2.3.4 Sex

Detweiler (1964) reported that incidence of congestive heart failure was six times as high as in males as females.
Cardiac diseases in male dogs were found to be 1.5 times more prevalent than in females (Häggström et al., 1994).

**Myocardial Diseases**

Tidholm and Jonsson (1996) reported in DCM that 62 per cent dogs were females and 38 per cent dogs were males. Tidholm et al. (1997) in a study of 187 dogs with DCM reported 116 males and 73 females. Calvert et al. (1997) had shown that there was a preponderance of male Dobermans to develop dilated cardiomyopathy.

O’Grady and Horne (1998) reported that in Doberman pinschers the DCM occurred approximately 67 per cent in males and 33 per cent in females.

Vollmar (1999) in Dilated Cardiomyopathy found that 30 were males and 36 were females in 60 animals. Vollmar (2000) in a survey of 500 wolfhound dogs reported DCM in 121 (24.2 per cent) dogs, among these, 65 were males and 56 were females.

Petric et al. (2002) in a study of 52 dogs of different breeds reported DCM in 42 males and 10 females and they also reported that in case of Doberman pinchers 16 were males and 5 were females. Borgarelli et al. (2006) in a study of 63 dogs with DCM reported that 57 dogs were male and 6 dogs were females.

Meurs et al. (2007) reported that there was no sex difference noticed in the occurrence of DCM in Doberman pinchers. Martin et al. (2009) in a study of 369 clinical cases of DCM observed that 73 per cent (281) of dogs were male and 27 per cent were female with a ratio of 2.7:1 (male: female).

Martin et al. (2010) in a study of 367 clinical cases of DCM observed that 263 (74.3 per cent) were male. Wess et al. (2010) found that there was an equal gender distribution in the occurrence of DCM, but male dogs showed earlier echocardiographic changes than female dogs.

**Valvular Diseases**

A greater predisposition of congestive heart failure was reported in male dogs owing to mitral valve insufficiency (Thrusfield et al., 1985).
Chronic atrioventricular valvular disease was seen more commonly in males compared to females (Detweiler and Patterson, 1965). Gooding et al. (1982) documented seven male dogs with both chronic mitral valve disease and congestive cardiomyopathy in a study report of eight dogs with congestive heart failure.

2.4 PHYSICAL EXAMINATION FINDINGS AND CLINICAL SIGNS

Fisher (1972) reported louder heart sounds, triple gallop rhythm, pale mucous membranes, cold extremities, systolic murmur, distress on exercise, tachypnoea or dyspnoea, coughing, gross overweight, harsh inspiratory and expiratory sounds, ascites, limb oedema, and weight loss in dogs with heart disease.

Bulmer (2006) stated that patients with severe chronic cardiac compromise might exhibit marked weight loss and muscle wasting along the temporal region of the head and the dorso-ventral aspect of the spine.

Erling and Mazzaferro (2008) stated that clinical signs of left sided congestive heart failure included respiratory distress, exercise intolerance, lethargy, anorexia, tachypnea and a moist, possibly productive, sometimes nocturnal cough.

Myocardial Diseases

Fox (1988) stated that the clinical signs for DCM often occurred acutely and include dyspnoea, coughing, syncope, exercise intolerance, and abdominal distension.

Tidholm and Jonsson (1996) in a study of 37 Newfoundland with DCM observed the following physical examination findings which include dyspnoea (100 per cent), systolic murmur (11 per cent), weak femoral pulses (61 per cent), ascites (35 per cent), weight loss (30 per cent) and elevation of body temperature above 39ºC (100 per cent).

Tidholm et al. (1997) in a study of 187 dogs with DCM observed systolic murmur in 48 dogs, dyspnoea in 108 dogs and ascites in 155 dogs, cough in 113 dogs, panting in 146 dogs, depression in 100 dogs, exercise intolerance in 165
dogs, inappetance in 122 dogs, polydipsia in 159 dogs, weight loss in 145 dogs, syncope in 157 dogs, and abdominal distension in 177 dogs.

McEwan (2000) observed cold extremities, weak femoral pulse, pale mucous membranes, gallop rhythm, systolic murmur, exercise intolerance, tachypnea or dyspnoea, coughing, shortness of breath and muscle wasting in DCM.

Meurs et al. (2001) in a study of 17 Great Dane with DCM observed coughing (8 dogs), exercise intolerance (6 dogs) and weight loss (5 dogs) as common clinical abnormalities.

Borgarelli et al. (2006) in a study of 63 dogs with DCM observed heart murmur in 48 dogs (76 per cent), gallop rhythm in 16 dogs (25 per cent), ascites in 17 dogs (27 per cent), and dyspnoea in 38 dogs (60 per cent).

Inappetance and anorexia, fatigue, dyspnoea, malaise, abdominal discomfort associated with ascitic distension and hepatomegaly, malabsorption and maldigestion, cardiac cachexia were most commonly seen symptom in DCM, especially with right sided congestive heart failure, which also remained as a poor prognostic indicator (Jordan, 2003).

Clinical signs of arrhythmogenic right ventricular cardiomyopathy included syncope, increased risk of sudden death, intermittent weakness and in some instances congestive heart failure (Baumwart and Meurs, 2005).

Tidholm and Jonsson (2005) identified pleural effusion and pulmonary oedema as independent prognostic indicators with dilated cardiomyopathy. The other clinical signs included arrhythmia, low-intensity systolic murmur, weak femoral pulse, ascites and gradual weight loss.

Martin et al. (2009) observed that the most common signs in DCM were breathlessness (67 per cent), cough (64 per cent), exercise intolerance (48.8 per cent), weakness (39.2 per cent), reduced appetite (35.7 per cent), weight loss (30.2 per cent), collapse (26 per cent), dullness and lethargy (20 per cent). They also found that there were some differences in the clinical presentation between breeds. Collapse (70 per cent) in boxers; reduced appetite (60 per cent) in Golden retrievers; weakness (55.3 per cent) in Great Danes were most common. However,
in many breeds, coughing and breathlessness were the dominant clinical signs at presentation. Exercise intolerance was common in German shepherd (GSDs) (61 per cent), Great Danes (58 per cent), Saint Bernards (58 per cent) and Irish wolfhounds (IWH) (53 per cent), and weakness was common in Great Danes (58 per cent), Saint Bernards (52 per cent) and GSDs (48 per cent).

**Valvular Diseases**

Komitor (1976) found that dogs with mitral valve disease were restless, unable to sleep and exhibited audible wheezing. Generalized muscle weakness, exercise intolerance, syncope and ascites were noticed in dogs affected with mitral regurgitation (Buchanan, 1977).

Bonagura and Frank (1983) observed that cough and dyspnoea, increased broncho vesicular sounds, hyperkinetic atrial pulses and cardiac murmurs in dogs with aortic valvular disease.

A weak femoral artery pulse was documented as a common finding in Cavalier King Charles Spaniels with valvular disease leading to heart failure (Beardow and Buchanan, 1993).

Häggström *et al.* (1995) reported that there was an association between auscultatory intensity of cardiac murmurs and the severity of mitral regurgitation. Hence the dogs might be screened for development and severity of chronic valvular disease by the use of cardiac auscultation.

Mitral regurgitation, the most prominent clinical finding is a systolic heart murmur. This heart sound originates from blood turbulence and associated vibrations that develop when ventricles eject blood in to the atrium (Swenson *et al.*, 1996).

Syncope was encountered in some dogs with chronic mitral valve insufficiency which might be associated with a tachyarrhythmia and the episode varied from occasional spells to several attacks per day (Kapoor, 1997).

French *et al.* (1998) reported that the dogs afflicted with mitral valve disease had wheeze and a hacking type of cough.
Kittleson and Kienle (1998) found that coughing was a common presenting complaint in dogs with a murmur of mitral regurgitation, which was exacerbated by excitement or exercise.

Palacio et al. (1998) described that reduced exercise tolerance, weakness or syncope occurred mainly in instances of pulmonary hypertension secondary to mitral valve insufficiency or tachyarrhythmia. Cough was the most prominent presenting complaint in chronic mitral valve insufficiency.

Kvart and Häggström (2000) stated that the most prominent clinical finding in aortic stenosis, regardless of its location, was a crescendo-decrescendo murmur located in the left heart base or over the right cranial thorax due to turbulence of blood flow.

Intense, markedly irregular heart sound on auscultation and murmur at left heart base and often dysrhythmia were noticed in dogs with aortic valve disease (Brown, 2004).

Ristic (2004) observed that exercise intolerance, soft honking cough was reported as one of the most common clinical complaints associated with heart failure whereas pale mucous membrane, weak pulse and pulse deficit were found to be associated with advanced stages of heart failure.

Linde and Koch (2006) in their study emphasized auscultation as a useful tool for screening of Boxers for potential cardiac disease including aortic stenosis.

### 2.5 Haematology and Serum Biochemistry

Tidholm et al. (1997) stated that evaluation of haematology and blood chemistry was important in dogs with DCM to exclude other primary or concurrent diseases; however there may be no abnormalities and further stated that increases in blood urea might be detected in some dogs, possibly a sign of prerenal azotaemia due to low cardiac output.

#### 2.5.1 Haematology

Lombard (1984) observed mild and poorly regenerative anaemia’s in two dogs with DCM. Tidholm and Jonsson (1996) observed no haematological abnormalities in Newfoundland dogs with DCM.
Ristic (2004) opined that haematology and biochemistry were not particularly useful for the diagnosis of heart disease; however, they could be helpful to investigate potential concurrent disease. He also opined that cardiac disease might have an elevated stress leukogram and an increase in mature neutrophil counts.

A low percentage of haematocrit, haemoglobin levels and increased leukocytes, neutrophils and platelets were recorded in dogs with congestive heart failure (Farabaugh et al., 2004) whereas Feliu-Pascual et al., (2006) recorded an average normal haematology in 25 Great Danes with cardiomyopathy.

Martin et al. (2009) recorded that the haematocrit value was low in 9.7 per cent in dogs with DCM.

2.5.2 Serum Biochemistry

In severe cardiac failure there was evidence of azotaemia with low total protein and albumin. This might be a dilutional effect of retained water, reduced protein synthesis or absorption. Elevated serum alanine transferase and serum alkaline phosphatase were noticed in animals with right-sided congestive cardiac failure. Serum bile acid levels were usually within normal range. Hypokalaemia, hypochloremia and hyponatremia were noticed in dogs with cardiac failure (Kaneko et al., 1999).

Although dogs were considered to be resistant to atherosclerosis associated with hypercholesterolemia, it had been found that with increased cholesterol concentration of above 750 mg/dl, there was a relative increase in the low-density lipoprotein particles that might predispose the animal to atherosclerosis (Christopher, 1997; and Thomason et al., 2007).

Andreoli (1999) reported that renal retention of sodium was the major reason for water retention which leads to oedema in chronic congestive heart failure.

Myocardial Diseases

Lombardö (1984) and Martin et al., (2010) observed that the most frequent biochemical abnormalities in dogs with DCM were azotaemia, creatininaemia,
hypoalbuminaemia, elevated liver enzymes like serum alkaline phosphatase (SAP) and serum glutamate pyruvate transaminase (SGPT).

Thomas (1984) observed elevated SGPT levels and normal SAP levels in dogs with congestive heart failure due to idiopathic cardiomyopathy. Ware et al. (1990) reported that dogs with Dilated Cardiomyopathy and decompensated heart failures had serum sodium concentration at or below the lower end of normal range.

Simpson et al. (1994) recorded hyponatremia and hypochloremia owing to excessive retention of free water in a Dachshund with right ventricular cardiomyopathy while increased alanine transferase activity was attributed to hepatic congestion secondary to right sided-heart failure.

Tidholm and Jonsson (1996) observed no serum biochemical abnormalities except mild elevation of blood urea nitrogen in Newfoundland with DCM.

McEwan (2000) reported elevated blood urea nitrogen and creatinine, increased alanine transferase (ALT) enzyme, and hypoproteinaemia as biochemical changes in dogs with DCM. Martin et al. (2009) found only small changes in serum urea and creatinine in DCM.

Valvular Diseases

Charles and Hamilton (1962) found a low total protein concentration of 4.8g/L in dogs with mitral insufficiency and heart failure.


2.6 RADIOGRAPHY

Silverman and Suter (1975) suggested that radiography should be taken in full inspiration.

Cardiomegaly, pulmonary oedema and aortic arch prominences were seen with thoracic radiography in dogs with aortic stenosis (Bonagura and Frank, 1983).
Echocardiographically normal size of left atrium was frequently diagnosed as enlarged by radiographs. These errors were due to shape changes of the left atrium; the radiographic criteria for left atrial enlargement might be too sensitive leading to false positive diagnoses. (Lombard et al., 1985).

The radiographic changes noticed in dogs with mitral regurgitation included marked left atrial enlargement, pulmonary congestion, enlargement of the caudal venacava and hepatic enlargement (Darke et al., 1996).

Tidholm and Jonsson (1996) in DCM were observed cardiomegaly (moderate to severe), pulmonary oedema, pleural effusion and ascites. Pulmonary oedema was the most common finding on thoracic radiographs of dogs with symptomatic DCM, whereas signs of right-sided heart failure i.e., pleural effusion, pericardial effusion and ascites were reported to be common in giant breeds with DCM (Tidholm and Jonsson, 1997; Vollmar, 2000).

Left atrial enlargement was most pronounced in dogs with congenital or acquired mitral regurgitation. In severe LA enlargement, left atrium becoming denser than the rest of the cardiac silhouette and appeared as a round radio dense region between the two main stem bronchi (Kittleson, 1998).

Left atrial enlargement was one of the earliest and most consistent radiographic feature of chronic mitral valve insufficiency. With progression, the left atrium and the left ventricle continued to enlarge (Kvart and Häggström, 2000).

Radiographic diagnosis was recommended as a preferable method for assessing evidence of left-sided heart failure and to identify generalised cardiac enlargement, specific cardiac chamber or great vessel enlargement, pulmonary parenchyma and vascular abnormality (Ruehl and Thrall, 1981 and McEwan, 2000).

Lamb et al. (2001) concluded that survey radiography was an inaccurate method for the diagnosis of canine congenital cardiac anomalies.

Meurs et al. (2001) observed pulmonary oedema, generalized cardiac enlargement, left atrial enlargement and pleural effusion as the common radiographic abnormalities with DCM.
MacDonald et al. (2003) observed caudo-dorsally distributed interstitial to alveolar pulmonary infiltrate and left atrial enlargement.

Tidholm and Jonsson (2005) had demonstrated that most common radiographic findings in dogs with dilated cardiomyopathy included cardiomegaly with prominence of the left atrium, pulmonary venous congestion and interstitial or alveolar pulmonary oedema.

Borgarelli et al. (2006) with DCM observed pulmonary oedema in 79 per cent and pleural effusion in 10 per cent in dogs by radiography.

On dorsoventral view, the left heart border usually became more convex and advanced toward the left chest wall, decreasing the space between the chest wall and the cardiac silhouette in dogs with mitral regurgitation. On lateral view, the caudal cardiac border became more rounded or straighter than normal (Lefbon, 2005).

Erling and Mazzaferro (2008) recommended radiography as the best way to document cardiogenic pulmonary oedema, which could be of help in evaluating the patient before the onset of heart failure.

Kraetschmer et al. (2008) suggested that the breed specific differences and the position of the animal (left or right lateral recumbency) should be considered while estimating cardiac size by means of vertebral heart score.

Martin et al. (2009) in DCM observed cardiomegaly in 80 per cent and congestive signs (pulmonary oedema or pleural effusion) in 74 per cent by thoracic radiography.

Ghadiri et al. (2010) concluded that it was important to consider the breed and the side of radiographic view when evaluating heart size in thoracic radiographs to avoid any erroneous interpretation of cardiac enlargement.

Martin et al. (2010) in DCM observed radiographic signs of cardiomegaly in 76.8 per cent and pulmonary oedema in 70.6 per cent.

**Vertebral Heart Scale**

Buchanan and Bucheler (1995) developed a method for measuring canine heart size in radiographs, vertebral scale system, on the basis that there was good
correlation between heart size and body length regardless of the conformation of the thorax. The lengths of the long and short axis of the heart were measured with callipers and the dimensions were scaled against the length of vertebrae, dorsal to the heart beginning, with fourth thoracic vertebrae (T4). The sum of the long and short axes of the heart expressed as vertebral heart size as 9.7±0.5 vertebrae.

Owens and Biery (1999) suggested simple radiographic thumb rules such as normal cardiac silhouette in the dog usually ranged from 2.5 to 3.5 times the width of the intercostal spaces.

Lister and Buchanan (2000) stated that the major importance of vertebral heart scale was to determine cardiomegaly in borderline cases with minimal radiographic changes and quantification of the progression of cardiomegaly over a period.

Lamb et al. (2000) concluded that there was little gain from measuring vertebral heart score (VHS). Lamb et al. (2001) recorded the vertebral heart scores for normal and healthy Labrador retrievers as 10.8. They concluded that the accuracy of VHS measurements for the diagnosis of cardiac disease varied with the breed.

Hansson et al. (2005) concluded that VHS method for heart size was independent of observer experience but dependent individual observer’s selection of reference points and transformation of long and short axis dimensions into VHS units.

Côté et al. (2013) concluded that thoracic radiographic findings should not be considered reliable for identification of dogs with Cardiac tamponade attributable to Pericardial Effusion.

2.7 SYSTOLIC BLOOD PRESSURE MEASUREMENT BY DOPPLER METHOD

Anderson and Fisher (1968) suggested that whichever method used for measuring blood pressure the animal should be unsedated, relaxed and minimally restrained. They also added that the heart rate should be within the normal range unless there was heart failure.
Valtonen and Eriksson (1970) stated that an oversized cuff might give erroneously low recordings, and an undersized cuff may give falsely high readings. They also suggested that for indirect blood pressure measurement the cuff width should be 40 per cent of the circumference of the limb.

Vulgamott and Clark (1980) recorded systolic hypertension (280 mmHg) in a Doberman pinscher with aortic valvular endocarditis and opined that insufficiency of the aortic valve (vegetative endocarditis) might be responsible for the systolic arterial hypertension.

Kittelson and Oliver (1983) stated that the blood pressure measured from the peripheral artery would not accurately reflect aortic pressure, because of significant peripheral resistance and pressure wave reflections combining to raise the systolic pressure. They also added that using correct cuff size was critical and the width should be 40 per cent of the circumference of the limb.

Harvey et al. (1983) reported that the normal blood pressure values by auscultatory method as systolic blood pressure (SBP) 145±25 mmHg and diastolic blood pressure (DBP) 84±14 mm Hg.

Coulter and Keith (1984) concluded that oscillometric method of indirect blood pressure measurement was more practically useful for detection of even subtle cardiovascular hemodynamic changes.

Cox and Bagshaw (1988) had shown that sustained experimental systolic hypertension resulted in increased arterial wall stiffness, especially with coronary arteries. Compensatory hypertrophy of heart attributable to increased afterload was reported in four out of five hypertensive dogs (Littmen et al., 1988).

Systemic vasoconstriction and stimulation of renin release led to increased blood pressure in heart failure in dogs (Ware et al., 1990). Douglas and Tallent (1991) opined that increased after-load associated systemic hypertension might lead to left ventricular hypertrophy, diastolic dysfunction and secondary valvular insufficiency.

Weiser et al. (1992) reported that the normal blood pressure value measured by Doppler method was 135±26/74±14 mmHg as SBP/DBP
Michell and Bodey (1994) recorded blood pressure in pet dogs and obtained the average normal blood pressure values of about 130±12 /85±8 mm Hg by indirect method. The Doppler flow meter had been shown to detect the pulse signal in low flow states more efficiently than other devices (Binns et al., 1995). Major limitations of Doppler BP measurement was is inability to diagnose diastolic hypertension (Henik, 1997).

Bodey and Michell (1996) reported that the normal systolic, diastolic and mean arterial blood pressure by doppler measurement for giant breed dogs were 121.3mmHg, 67.1mmHg, and 90.5mmHg, respectively and for retrievers were 122.7mmHg, 69.2mmHg, and 91.2mmHg, respectively. They also reported in dogs with cardiac disease the normal systolic, diastolic and mean atrial blood pressure were 136.4mmHg, 77.9mmHg, and 101.4mmHg, respectively.

Branson et al. (1997) noticed that there was no significant difference in the blood pressure measurement obtained from thoracic limb and pelvic limb.

Stepien and Rappoport (1999) extensively reviewed various methods of blood pressure measurements and stated that Doppler ultra sound method was more consistent in detecting low pressure than other techniques.

Systemic hypertension had been associated with cardiovascular changes and many hypertensive dogs and cats with signs of laboured breathing were found to be related with heart failure (Brown and Henik, 1998). The authors reported that left ventricular hypertrophy might regress with control of systemic hypertension. The dogs with arrhythmia had beat-to-beat variation in the strength of the cardiac contraction (Carr, 2001).

Henik et al. (2005) considered non-invasive blood pressure measurements as the standard diagnostic test in small animal clinical practice.

De-Morais and Schwartz (2005) opined that arterial blood pressure should be measured in all patients with heart disease or congestive heart failure. Arterial hypertension could lead to left ventricular hypertrophy and dysfunction and eventually to congestive heart failure.

2.8 ELECTROCARDIOGRAPHY (ECG)
The common electrocardiographic abnormalities in dogs with heart failure included left ventricular hypertrophy, left atrial enlargement, left bundle branch block and atrial fibrillation (Gooding et al., 1982).

Sinus rhythm with first degree atrioventricular block and variable periods of second degree AV block were noticed in Boxer with aortic stenosis (Bonagura and Frank, 1983).

Brownlie (1991) in a study associated with Iris Wolfhounds reported atrial fibrillation in 46 per cent dogs, ventricular premature contractions in 13 per cent dogs, supraventricular premature contractions in 10 per cent dogs, first and second degree AV block in 8 per cent dogs and P-mitral in 10 per cent dogs.

Brownlie and Cobb (1999) observed the following electrocardiography (ECG) abnormalities in Iris Wolfhounds, Atrial fibrillation 11.6 per cent, supraventricular premature contractions in 3.3 per cent, ventricular premature contractions in 6.1 per cent, supraventricular tachycardia in 1 per cent, first degree atrioventricular block 4.5 per cent, right bundle branch and left fascicular blocks in 8.3 per cent.

McEwan et al. (2003) observed atrial fibrillation as the most common ECG findings in large and giant breed dogs. The other findings were supraventricular premature complex, VPC’s, and ventricular tachycardia.

**Myocardial Diseases**

Atrial fibrillation was the most commonly diagnosed electrocardiographic abnormality (Tilley and Liu, 1975 and Fox, 1988) although ventricular premature depolarization and ventricular tachycardia were reported in a majority of Doberman Pinschers with DCM. (Calvert et al., 1982; Calvert and Brown, 1986).

In an electrocardiogram study of Cocker Spaniels with cardiomyopathy, Gooding et al. (1986) diagnosed left ventricular enlargement, biventricular enlargement, deep Q waves and right ventricular hypertrophy, left atrial dilatation. Sisson (1987) reported QRS complexes of abnormally high amplitude and left ventricular enlargement in dogs with sub aortic stenosis.
Sisson and Thomas (1995) observed atrial fibrillation in 75 to 80 per cent of giant breed dogs with DCM. Tidholm and Jonsson (1996) in a study of with DCM reported atrial fibrillation, and chamber enlargement, which included left atrial enlargement and left ventricular enlargement; and also heart rate ranged from 162 to 260 per minute (mean 204 per minute). Atrial fibrillation (AF) and Ventricular premature complexes (VPC) were documented to be common arrhythmias in dogs with DCM (Kittleson, 1998; Sisson et al., 1999; Calvert and Wall, 2001)

Calvert et al. (1997) recorded ventricular premature complex as a major electrocardiographic change in dogs with occult cardiomyopathy. McEwan (2000) opined that ventricular arrhythmia might be present up to nine months prior to development of echocardiographic evidence of dilated cardiomyopathy in Doberman Pinschers.

Simpson et al. (1994) recorded a prominent S wave suggestive of right ventricular enlargement and sinus rhythm with left axis deviation in a Dachshund with right ventricular cardiomyopathy.

Vollmar (2000) reported atrial fibrillation in 23.4 per cent and rhythm disturbances in a study of Irish wolfhounds with Cardiomyopathy. McEwan (2000) stated that the presence of atrial fibrillation (AF) on ECG was the usual sign of Dilated Cardiomyopathy in Newfoundland.

Meurs et al. (2001) in a study of Great Danes with DCM, observed atrial fibrillation and ventricular premature complex.

Great Danes affected with DCM often have atrial fibrillation along with other clinical signs (Menaut et al., 2005; Martin et al., 2009).

Tidholm and Jonsson (2005) found atrial fibrillation and ventricular arrhythmia as predominant abnormalities on electrocardiogram recording in dogs with dilated cardiomyopathy.

Borgarelli et al. (2006) observed isolated supraventricular arrhythmias in 3 per cent, isolated VPC, and ventricular tachycardia in DCM cases. Billen and van Israel (2006) reported that atrial fibrillation, ventricular premature complexes and
ventricular tachycardia were common arrhythmias in dogs with Dilated Cardiomyopathy associated with syncope.

Billen and Israël (2006) opined that there was a possibility of transient atrioventricular block causing syncope in dogs with Dilated Cardiomyopathy and atrial fibrillation.

Martin et al. (2009) in a study of DCM recorded atrial fibrillation (AF) in 45 per cent, ventricular premature complexes (VPCs) in 31 per cent and supraventricular premature complexes (SVPCs) in 9 per cent of dogs. AF was most common in the giant and large breed dogs which included Irish wolfhounds (94.1 per cent), Great Danes (78.9 per cent), Newfoundlands (77 per cent), GSD (73 per cent) and Saint Bernards (72 per cent) and least common in Cocker Spaniels (8 per cent). VPCs were most common in Boxers (52.8 per cent) and Dobermans (44 per cent). SVPCs were most commonly seen in boxers (25 per cent) and GSDs (14 per cent).

Martin et al. (2010) in DCM observed that the electrocardiographic abnormalities such as atrial fibrillation in 43.5 per cent, supraventricular premature complexes in 8.5 per cent and ventricular premature contractions in 29.9 per cent.

Wess et al. (2010) concluded that a 5-minute ECG was a rather insensitive method for detecting arrhythmias in Doberman pinschers. However, the occurrence of at least one VPC in five-minutes strongly warrants further examination of the dog that could suggest occult cardiomyopathy.

**Valvular Diseases**

Electrocardiogram had a low sensitivity in detecting right atrial and right ventricular enlargement secondary to primary tricuspid valve insufficiency. In significant tricuspid valve insufficiency with pulmonary hypertension, the electrocardiographic changes might include evidence of right atrial enlargement and right ventricular enlargement (Tilley, 1992).
The presence of sinus arrhythmia in advanced chronic mitral valve insufficiency indicated that heart failure was absent. Rapid and irregular heart rate was indicative of arrhythmia. The most common form of ectopy was atrial premature contractions. In progressed chronic mitral valve insufficiency, supraventricular tachycardia, atrial fibrillation and ventricular premature contractions can be encountered (Häggström et al., 1992).

Darke et al. (1996) reported electrocardiographic abnormalities associated with mitral valve diseases. The changes included widened P and R waves with increased amplitude, atrial premature complexes and atrial fibrillation. Electrocardiographic findings in chronic mitral valve disease varied from normal tracings to marked abnormalities in rate, rhythm and configuration of complexes.

With the exception of documenting and classifying certain arrhythmias, the electrocardiogram was of limited use in the diagnosis or management of chronic mitral valve disease (Kittleson, 1998).


Hyun (2005) documented P-pulmonale, P-mitrale and sinus arrhythmia in a Cavalier King Charles Spaniel with mitral valve prolapse.

2.9 ECHOCARDIOGRAPHY

Borrow et al. (1980) concluded that end systolic volume index was a more accurate parameter of systolic dysfunction because it was mainly dependent on afterload and contractility and was relatively independent of preload. Values >30ml/ m² were considered to indicate systolic dysfunction.

Pipers (1981) advocated the use of echocardiography to detect abnormalities of the mitral valve in dogs.

Boon et al. (1983) concluded that there was a linear relation between increasing body size and increasing heart size; but none of the indices of left ventricular function, mitral valve motion velocities, or dimension ratios showed significant correlation to body surface area.
Wingfield et al. (1982) concluded that left lateral supine positioning of the dog improved echocardiography imaging. Normal values of canine echocardiogram were established in veterinary cardiology and these measurements were useful to assess abnormal cardiac dimensions with mitral regurgitation (Lombard et al., 1984; Boon, 2006).

De Madron et al. (1983) studied normal and paradoxical ventricular septal motion in the dog and suggested that abnormalities in ventricular septal motion should cause a clinician to suspect right ventricular volume and pressure overload.

Kittleson et al. (1984) in their study on myocardial function in small dog with mitral regurgitation and congestive cardiac failure found that end diastolic volume index increased linearly with end systolic volume index indicating that myocardial failure resulted in further salt and water retention.

M-mode echocardiogram particularly the LA/Ao ratio was very sensitive and useful for an early detection of left atrial enlargement in dogs (Lombard et al., 1985). Nishimura et al. (1985) stated that the functional analysis of cardiac structure could be obtained by M-mode and two dimensional echocardiography.

Calvert and Brown (1986) reported that M-mode echocardiography provided a non-invasive method of evaluating cardiac chamber size, interventricular septum, left ventricular free wall thickness, systolic and diastolic functions.

Nodular thickening and rupture of chordae tendinae could be observed in dogs with mitral regurgitation by use of M or B mode echocardiography. But the technology had been limited to diagnostic purposes for the valvular diseases, probably for the reason that it was difficult to quantify the mitral apparatus in motion with rapid heart rate (Sisson, 1987).

Jacobs and Mahjoob (1988) adopted multiple regression analysis in dogs using body size and cardiac cycle length in predicting echocardiographic variables and found positive correlation between left ventricular internal chamber dimension in diastole and systole, body weight, body surface area, cycle length and square root of cycle length. The shortening fraction had a significant negative
correlation and left ventricular free wall measurement had a significant positive correlation to body weight and body surface area.

Brown et al. (1991) observed that significant correlations existed between body weight and estimated left and right ventricular stroke volume and cardiac output.

Crippa et al. (1992) concluded from their study that there was no correlations existed between echocardiographic parameters and animal size. They also suggested that cardiac morphological and functional homogeneity existed for the particular breed and age without any precise relation with body weight.

Kittleson (1998) recorded the echocardiographic changes in dogs with severe mitral regurgitation. The changes included moderate to severely enlarged left atrium, LA/Ao ratio greater than 2.0, moderately enlarged left ventricle, fractional shortening of greater than 50 per cent.

The consequences of aortic regurgitation caused by bacterial endocarditis demonstrated through echocardiographic changes included diastolic flutter of mitral valve, left ventricular dilatation, ventricular hyperkinesia and premature closure of mitral valve (Kvart and Häggström, 2000).

McEwan et al. (2003) observed the following echocardiographic signs in dogs with DCM; left ventricular dilation, reduced systolic function and increased sphericity of the left ventricle.

O’Leary et al. (2003) reported that normal echocardiographic parameters varied between breeds as do body size. Echocardiographic reference ranges derived from some dog breeds might be misleading for others. Breed specific or at least somatotype specific normal echocardiographic parameters such as left ventricular, atrial measurements and aortic volume were required to make more accurate cardiac diagnosis and assess disease severity.

Lombard (1984) and Cornell et al. (2004) reported that the various variables that could influence echocardiographic evaluation of systolic function include age, sex, breed, weight and co morbid factors (hypothyroidism and hydration factors).
Cote (2005) described various pitfalls arising during echocardiographic imaging and suggested practical solutions to overcome the pitfalls.

Teshima et al. (2005) suggested that canine pulsed tissue Doppler imaging could be clinically applied for the estimation of cardiac function, detection of cardiac decompensation and left atrial volume overload in dogs with mitral regurgitation.

Teshima et al. (2006) assessed left ventricular Tei index (index of myocardial performance) in healthy dogs and dogs with mitral regurgitation and found that there was increase in left ventricular Tei index with the progression of clinical signs in dogs with mitral regurgitation.

Kayar et al. (2006) in their study on M-mode echocardiographic parameters and indices in the normal German shepherd dog found that there was significant association between body weight and inter ventricular septum, left ventricular internal diameter and left ventricular posterior wall thickness in systole and diastole, fractional shortening, left atrial dimension, aortic root dimension, right ventricular internal dimension, D-E amplitude and D slope of the mitral valve.

Echocardiography was a useful tool for non-invasive evaluation of systolic function. Most of the indices commonly used to evaluate systolic function such as fractional shortening, ejection fraction in systolic volume index were strictly dependent on intrinsic contractility, preload, afterload and wall stress. Therefore any variation in these indices must take into account while interpreting these factors (Borgarelli et al., 2007).

Chetboul and Tissier (2012) reported conventional two-dimensional, M-mode, and Doppler examination plays a critical role in the initial and longitudinal assessment of dogs affected by MVD, providing information on mitral valve anatomy, MR severity, left ventricular (LV) size and function, as well as cardiac and vascular pressures. Several standard echocardiographic variables like left atrium to aorta ratio, regurgitation fraction and pulmonary arterial pressure may also help in identifying asymptomatic MVD dogs during higher risk of early decompensation.
2.9.1 Two-Dimensional Echocardiography

Quinones et al. (1981) opined that teichholz method (M-mode method) of calculating left ventricle (LV) volumes and ejection fraction (EF) from LV, linear dimensions might result in inaccuracies because of the geometric assumptions required to convert linear measurement to three dimensional volumes.

Thomas (1984) concluded that complete anatomic evaluation of the canine heart can be obtained by 2-D echo in normal dogs from standardized transducer locations. Calvert and Brown (1986) reported the average two-dimensional echo values of healthy Doberman for left atrium (LA) as 26.63±1.5 mm and aorta (Ao) as 29.9±2.31mm.

Schiller (1991) opined that the modified Simpson’s rule, which was recommended by American Society of Echocardiography was the most commonly used formula for volume determination in humans. Koch et al. (1996) reported the normal two-dimensional echo values of healthy Great Dane for LA as 33.0 mm with the range of 28.0 - 46.0 mm and Ao as 29.5 mm with the range of 28.0 – 34.0 mm and of healthy Iris Wolfhound for LA as 31.0 mm with the range of 22.0 - 36.0 mm and Ao as 30.0 mm with the range of 29.0 – 33.0 mm and of healthy Newfoundland for LA as 30.0 mm with the range of 24.0 - 33.0 mm and Ao as 29.0 mm with the range of 26.0 – 33.0 mm.

Vollmar (1996) reported the average two-dimensional echo values of healthy Iris Wolfhound for LA as 33.7 ± 5.9 mm and Ao as 30.5 ± 4.0 mm. Boon (2011) observed that the formulas used in modified Simpson’s rule had best correlation with actual left ventricular volumes in the diseased heart and appeared relatively unaffected by changes in ventricular geometry.

McEwan (1999) reported the average two-dimensional echo values of healthy Newfoundland for LA as 24.13±4.06mm and Ao as 29.18±2.71mm. Vollmar (1999) observed the following two dimensional echocardiography values in normal Irish wolfhounds LA - 47.3 mm, RA - 40.4 mm, RVIDd - 29.1 mm and for Irish wolfhounds with advanced stage of DCM; LA - 71.2mm, RA - 47.8mm and RVIDd - 36.6 mm. McEwan (2002) opined that maximal left ventricular (LV) lengths and volumes were usually imaged from right parasternal long axis views.
compared with left apical views. McEwan et al. (2003) suggested that less than 40 per cent ejection fraction (EF) determined by 2D echocardiographic images (modified Simpson’s rule) from the right parasternal long axis four chamber view was abnormally low.

Lang et al. (2006) opined that M-mode methods of volume determination sometimes had a poor correlation with non-invasive methods and may not be clinically useful in the presence of heart disease. He also added that the volume determination by 2D echocardiographic images seemed to be more accurate, because they involve more direct measurements and fewer geometric assumptions.

Borgarelli et al. (2006) in DCM the mean (SD) of B-mode measured EF was 27.7 per cent (13.4 per cent) and the mean (SD) LA/Ao ratio was 2.1 (0.64). Kayar et al. (2006) reported the average two-dimensional echo values of healthy German Shepherd Dog for LA as 24.6±2.3 mm and Ao as 27.1±1.8 mm.

Serres et al. (2008) opined that either right parasternal long axis view or left apical 4-chamber view could be used for Simpson’s method of disc (SMOD) for measuring chamber volume.

Wess et al. (2010) conducted a study to establish reference values for Simpson’s method of disc and compared with M-mode measurement and opined that Simpson’s method of disc measure of End diastolic volume index (EDVI) and End systolic volume index (ESVI) was superior to conventional M-mode measurements in its ability to detect early echocardiographic changes is Doberman pincher with DCM.

### 2.9.2 M-Mode Echocardiography

Koch et al. (1996) reported the normal M-mode echo values for healthy Newfoundland. They were LVIDd – 50.0 mm with the range of 44.9 – 60.0 mm, LVIDs -35.5 mm with the range of 29.0 – 44.00 mm, LVPWd -10.0 mm with the range of 8.0 – 13.0 mm, LVPWs – 15.0 mm with the range of 11.0 – 16.0 mm, IVSd – 11.5 mm with the range of 7.0 – 15.0 mm, IVSs –15.0 mm with the range of 11.0 – 20.0 mm, RVIDd– 19.0 mm with the range of 6.0 – 28.0 mm, FS – 30.0 per cent with the range of 22.0 – 37.0 per cent.
McEwan (1999) reported the average M-mode echo values of healthy Newfoundland for LVIDd as 45.35 ± 4.03 mm, LVIDs as 34.31 ± 3.0 mm, LVPWd as 10.28 ± 1.13 mm, LVPWs as 13.69 ± 1.38 mm, IVSd as 10.66 ± 1.13 mm, IVSs as 12.93 ± 1.38 mm, RVIDd as 8.3 ± 3.0 mm and FS as 24.47 ± 3.21 per cent.

Vollmar (1999) observed the following M-mode echocardiography values in normal Irish wolfhounds which included LVIDs - 35.4 (2.8) mm, LVIDd- 53.2 (4.0) mm, FS - 34.0 (4.5) per cent, LVPWs - 14.9 (2.2) mm, LVPWd- 9.8 (1.6) mm, IVSs - 13.7 (2.4) mm, IVSd- 9.3 (1.8) mm, EPSS - 6.8 (1.6) mm and for Irish wolfhounds with advanced stage of DCM which included LVIDs - 52.4 (8.0) mm, LVIDd- 67.4 (6.2) mm, FS - 20.7 (8.3) per cent, LVPWs - 13.5 (3.6) mm, LVPWd - 8.9 (2.3) mm, IVSs - 12.4 (3.5) mm, IVSd- 47.3 (11.1) mm and EPSS - 13.4 (5.2) mm.

Calvert et al. (2000) suggested that in Doberman pinschers, left ventricular diastolic internal dimension (LVIDd) greater than 45 mm in dogs weighing less or as much as 42 kg, and (LVIDd) greater than 49 mm in dogs weighing over 42 kg, were abnormal.

Schober and Baade (2000) compared the left ventricular (LV) M-mode echocardiographic indices derived from right parasternal long axis and short axis imaging planes and concluded that the difference between two methods were small and within clinically acceptable limits in normal dogs.

Tilley et al. (2008) suggested the following M-mode values for healthy dogs weighing 30 kg. They were LVIDd- 48.3 ± 3.9 mm, LVIDs - 33.9 ± 3.4 mm, LVPWd - 8.4 ± 1.3 mm, LVPWs – 13.0 ± 1.3 mm, IVSd – 9.2 ± 1.3 mm and IVSs – 13.9 ± 2.3 mm.

Myocardial Diseases

Calvert et al. (1982) reported that M-mode abnormalities in DCM dogs which included increased left atrial dimension, increased left ventricular systolic and diastolic internal dimensions, decreased left ventricular fraction shortening, increased E-point septal separation (EPSS) and increased right ventricle internal dimension if the right side heart is involved.
Lombard\textsuperscript{a} (1984) compared the echocardiographic parameters with body weight and found that the left ventricular internal dimension in systole and diastole, the left ventricular wall thickness, the aortic root dimension and the left atrial dimension had high correlation coefficient ($r^2$) ranging from 0.756 to 0.619 with the body weight. The fractional shortening of the left ventricle in systole (39 per cent $\pm$ 6 per cent) and the left atrial to aortic root ratio (0.99 $\pm$ 0.10) were not linearly related to body weights and had constant values. He concluded that M-mode echocardiography was proved to be a useful technique in recognizing left heart dilatation and poor contractile function in dogs with Dilated Cardiomyopathy and congestive heart failure.

Calvert and Brown (1986) suggested that E-Point septal separation was determined to be the most sensitive and specific criterion for the recognition of early cardiomyopathy. The diagnostic criteria for DCM were decreased fractional shortening (FS), severe left atrial and ventricular dilatation without other detectable cardiac abnormalities (Thomas, 1987; Fox, 1989; Keene, 1989).

Atkins and Snyder (1992) reported that echocardiographic evaluation of left ventricular systolic performance revealed increased end-systolic and end-diastolic dimensions, dilatation of the left atrium, and decreased fractional shortening in DCM.

Monnet \textit{et al.} (1995) opined that diagnosis of DCM was based on the following findings which included increased left ventricular end systolic dimension (ESD) and end-diastolic dimension (EDD), decreased fractional shortening (FS), and increased E-point septal separation (EPSS).

In DCM, Tidholm and Jonsson (1996) recorded left ventricular hypokinesis in 100 per cent of cases, with FS ranging from 5 to 22 per cent, left ventricular end diastolic diameter (LVEDD) ranged from 5.0 to 8.4 cm (reference range, 3.0 to 6.0 cm in the healthy controls), and left ventricular end systolic diameter (LVESD) ranged from 4.3 to 8.0 cm (reference range, 2.2 to 4.4 cm in the healthy controls). They also stated that there was more overlap between the sick and healthy dogs in the distribution of LVEDD values than in LVESD values.
Borgarelli et al. (1997) observed that except FS or E-point septal separation (EPSS) all other echocardiographic indices of left ventricular volume were not proved to be good prognostic markers in dogs with DCM.

Echocardiographic evaluation of left ventricular systolic performance in dogs with DCM revealed increased end-systolic and end-diastolic dimensions, dilatation of the left atrium, and decreased fractional shortening (FS) less than 15 per cent, as well as changes in systolic time intervals (Calvert et al., 1997).

Kittleson (1998) categorized the severity of DCM based on the M-mode derived fractional shortening as mild (20-25 per cent), moderate (15-20 per cent) and severe (less than 15 per cent).

McEwan (2000) suggested the following M-mode echocardiography criteria for the diagnosis of DCM. They were large left ventricular lumen (particularly during systole), thin interventricular septum or left ventricular posterior wall, FS less than 20 per cent and mitral EPSS more than 10 mm for any breed.

Vollmar (2000) considered the following echocardiographic criteria for the diagnosis of DCM in Irish wolfhounds. They were LVIDd greater than 61.2 mm, LVIDs greater than 41 mm, FS below 25 per cent, EPSS greater than 10 mm, RVIDd greater than 36.8 mm and systolic internal dimension of atrium greater than 56 mm.

Petric et al. (2002) reported the following M-mode echocardiography values in Doberman pinchers affected with DCM as LVIDd -70.5±9.0 (54 to 83) mm, LVIDs - 64.6±9.8 (44 to 79) mm, FS -8.5±5.7 (14 to 18) per cent, LVPWd -8.42±1.3 (7 to 11), LVPWd -10.2±2.2 (6 to 12.6), IVSd -8.8±2.2 (6 to 12.3) and IVSs -11.2±3.4 (6 to 16).

McEwan et al. (2003) proposed the following guidelines for the diagnosis of DCM:

1. Left ventricular dilation (especially in systole but also in diastole).
2. Depressed systolic function.
3. Altered geometry of the left ventricle (increased sphericity).
4. Left or bi-atrial enlargement
5. M-mode fractional shortening of < 20 per cent or 25 per cent (depending on breed-specific reference values).
6. Left ventricular ejection fraction less than 40 per cent.
7. Increased mitral valve M-mode E point to septal separation (EPSS).

O’Grady and O’Sullivan (2004) stated that the echocardiographic abnormalities of DCM consisted of left ventricular (LV) enlargement in systole and/or diastole that would progress to clinical phase with signs of left sided congestive heart failure. He also reported that Fractional shortening (FS) was the major indicator of systolic function in veterinary echocardiography. Values of FS between 20 per cent and 25 per cent in Doberman pinschers were considered equivocal findings, but FS less than 15 per cent suggested strong evidence of DCM, although values from 18 per cent to 22 per cent had been observed in normal Doberman pinschers.

Borgarelli et al. (2006) in a study of 63 dogs with DCM the mean (SD) of M-mode FS was 16.4 per cent (7.7 per cent).

Kayar et al. (2006) reported the average M-mode echocardiography values of healthy German shepherd dog. They were LVIDd – 49.5±4.7mm, LVIDs - 34.3±3.4 mm, LVPWd -9.5±1.2 mm, LVPWs – 13.6±1.1 mm, IVSd – 9.8±1.4 mm, IVSs – 14.2±1.6 mm, RVIDd– 13.5± 0.9mm and FS – 31.4±3.4 per cent.

Chetboul et al. (2007) found that LV contractility along both the short and long axes was impaired in dogs with spontaneous DCM, as is systolic RV and diastolic LVFW function and these myocardial alterations are associated with an inverse force-frequency relationship.

Wess et al. (2010) considered LVIDd greater than or equal to 49 mm and LVIDs greater than or equal to 40mm as an indicator of DCM in Dobermans. Boon (2011) concluded 7.7 mm as normal value of EPSS in dogs.

Liu (1977) reported that asymmetric septal hypertrophy was the most common form of hypertrophy in dogs with HCM with 80 per cent of 20 dogs.
Atkins and Snyder (1991) reported FS in HCM is typically increased, and heart is very visibly hyperdynamic. In end stage HCM, systolic dysfunction can be identified before FS and EF decrease (Nagueh and Mahmarian, 2006).

Valvular Diseases

Lombard et al. (1985) in the study on correlation of radiographic, echocardiographic and electrocardiographic features of left heart enlargement in dogs with mitral regurgitation found that left ventricular hypertrophy patterns of the electrocardiography did not correlate either with radiographic diagnosis of left ventricular enlargement or with echocardiographic enlargement ratios. The incidence of P-mitrale was 30 per cent but this electrocardiographic abnormality when present, reliably identified enlarged left atrial dimension.

Chetboul and Tissier (2012) concluded that conventional echo-doppler examinations provides an accurate diagnosis of canine MVD and of its consequences on cardiac remodelling, LV function and cardiovascular pressures and they recommended serial echo-doppler examinations in dogs with MVD in order to identify and track these alterations over time and detect an ongoing worsening of the disease.

Pericardial effusion: Hoit (2007) reported that M-mode images show an echo-free space between the left ventricular wall and the pericardial sac. The epicardium on these images was very bright simply because of the difference in acoustical impedance between the epicardium and fluid. When the fluid space was not seen during systolic contraction of the LV chamber, the fluid accumulation was not clinically significant. The diastolic collapse of the RV secondary to tamponade was displayed on M-mode images as downward motion of the right ventricular wall during diastole. This was a sensitive and specific finding.

2.9.3 Colour Flow Doppler Echocardiography

Nishimura et al., (1985) and Smith et al., (1986) stated that colour flow Doppler echocardiography gave a physiological assessment of blood flow within cardiac chambers, across valve orifices, and in the great vessels. Kienle and Thomas (1995) opined that the Doppler technique provided only limited
additional information in dogs with dilated cardiomyopathy and said that the presence of severity of valvular insufficiency can be determined.

Diastolic mitral regurgitation using Doppler echocardiography in human beings with a variety of conditions including atrioventricular abnormalities, severe mitral regurgitation, aortic regurgitation, and hypertrophic cardiomyopathy had been reported by several authors (Panidis et al., 1986 and Vandenbossche and Englert, 1987).

Atkins and Snyder (1992) reported that systolic time interval offered a sensitive, useful and readily available tool for evaluation of cardiac function. They suggested that it could be used to diagnose and estimate the severity of myocardial function. Systolic time intervals were affected by variables like heart rate, loading conditions and contractility.

Numerous objective parameters of cardiac function could be derived from spectral Doppler tracings. These included maximal and mean flow velocities from which pressure gradient across stenosis, shunts and cardiac valves could be derived together with indices of ventricular systolic and diastolic function, flow volume and cardiac output (Darke et al., 1993).

Rosenthal and Fox (1995) detected diastolic mitral regurgitation in dogs and cats with atrioventricular block by pulsed wave Doppler echocardiography and colour flow Doppler mapping.

Kienle and Thomas (1995) stated that colour flow doppler was a further development of pulsed wave doppler in which colour-coded images of blood flow velocities could be superimposed on 2-D or M-mode images.

Darke et al. (1996) demonstrated colour flow Doppler recording from the mitral valve of a dog with mitral stenosis. The convergence of blood into the narrowed orifice resulted in an alias from red to blue at the region of proximal flow convergence. A ‘candle flame’ jet of diastolic turbulence was observed in the left ventricular inlet as blood enters the ventricle at high velocity.

Mitral regurgitation might be detected and quantified by spectral or colour flow Doppler ultrasound. The regurgitant flow should be aligned to ultrasound beam and this was most often achieved in the left apical four chamber view.
Spectral Doppler mapping might be used to identify the regurgitant jet while colour Doppler mapping was not available (Kittleson, 1998).

Certain qualitative blood flow characteristics such as laminar flow versus disturbed flow patterns and the abnormal timing and location of blood flow were easily recognized using Doppler echocardiography. Other variables such as flow velocity, volumetric flow rate and ejection time were made quantitative by combining two dimensional echocardiography and Doppler echocardiography. Quantitative information pertaining to cardiac responses to disease or therapeutic intervention could be estimated (Kvart and Häggström, 2000).

Abbott and Mac Lean (2003) compared Doppler derived aortic peak velocities obtained from sub costal and apical transducer sites in healthy dogs and reported that peak aortic velocity obtained from sub costal site exceeded those obtained from cardiac apex but did so only to a marginal degree. The results suggested that the diagnostic implication of sub costal and apical velocities were similar.

Colour M-mode imaging superimposed a colour flow Doppler study over a conventional M-mode study permitting the study of blood flow in relation to the anatomic structure. Unlike the two dimensional colour flow study which was displayed as a series of images run over time, the use of M-mode permitted the display of blood flow over time on a single image (Oyama, 2004).

2.10 TISSUE DOPPLER IMAGING (TDI)

2.10.1 TDI modes

As for conventional Doppler imaging, different TDI modes may be used: pulsed wave mode, 2D colour TDI mode and colour M-mode. Each one has its own specific advantages and drawbacks (Chetboul, 2002).

Chetboul (2010) demonstrated that TDI allowed early detection of systolic as well as diastolic myocardial dysfunction.

TDI includes several new techniques, such as tissue velocity imaging (TVI), strain, and strain rate that might be used to detect early myocardial dysfunction (Simak et al., 2011).

2.10.2 Pulsed wave TDI mode
Nagueh et al., (1997) observed that Em behaves as a preload-independent index of LV relaxation.

Garcia et al., (1998) reported that all pulsed Doppler indices of transmitral and PV flow present a parabolic distribution during progression from normal to advanced diastolic dysfunction and TDI have been shown to provide an accurate estimate of LV relaxation and appear to be relatively insensitive to the effects of preload compensation.

The pulsed wave TDI mode provides information on myocardial movements through a single sample volume. This sample volume is placed within the myocardial wall thickness using either a right parasternal short axis view or a left parasternal long axis view (Gavaghan et al., 1999) in order to analyse the circumferential or the longitudinal myocardial motion, respectively. The pulsed wave velocity profile includes, after a short isovolumic contraction phase, one positive systolic wave and after a short isovolumic relaxation phase, two diastolic negative waves (one called E and one called A, in early and late diastole respectively, with E/A ratio > 1). With pulsed wave TDI mode, velocity analysis is limited to the specific region where the sample volume is placed.

Chetboul (2002) reported that TDI enables global and regional myocardial velocities to be quantified from measurements of myocardial velocities in real time. Annular velocities can also be quantified using TDI technique.

Teshima et al. (2005) stated that E’ was lower in dogs with MR than in dogs without cardiac diseases and pulsed TDI can be applied clinically for estimation of cardiac function and detection of cardiac decompensation and left atrial volume overload in dogs with MR.

O’Sullivan et al., (2007) reported the values of PW TDI in Doberman in normal were Em:Am – 1.2-1.7, Sm(cm/s) – 17.3-21.9; in Occult DCM were Em:Am – 1.6-2.2, Sm(cm/s) – 9.4-11.9; in overt DCM were Em:Am – 2.2-3.2 Sm(cm/s) – 6.6-8.5 as diastolic function.

Hori et al. (2007) observed that in healthy dogs, the VS-TDI–derived S’ velocity and myocardial performance index (MPI) appear to be reliable assessments for evaluating LV systolic function.
Kobar et al., (2009) found that the combination of Doppler tissue echocardiography of the mitral septal annulus and mitral inflow patterns by conventional Doppler indices provides better estimates of diastolic dysfunction in dogs.

Tidholm et al. (2009) observed that in MVD animals 2 systolic velocities and 3 diastolic velocities were significantly increased in dogs with CHF compared with dogs without CHF and control dogs and Intraventricular dyssynchrony may be an early sign.

Chetboul and Tissier (2012) reported that tissue Doppler imaging provides new parameters to assess regional and global myocardial performance (e.g., myocardial velocities and gradients, deformation and rate of deformation, and mechanical synchrony) in degenerative mitral valve disease.

2.10.3 2D Colour Mode

Chetboul (2002) reported that the 2D colour TDI mode gives the best “overall impression” of myocardial movements because all velocity data are displayed simultaneously on the screen. As with conventional Doppler system, tissue velocities towards the transducer are coloured in red, and away from the transducer are coloured in blue. As with the pulsed wave TDI mode, the right parasternal short axis view and the left parasternal long axis view (4 or 5 chambers) allow the analysis of the transverse and longitudinal myocardial motions, respectively. With some ultrasound machines, after storage of 2D colour TDI loops, an off-line analysis of mean velocities was possible in separate myocardial segments (Garot et al., 1999; Hatle and Sutherland, 2000).

Estrada and Chetboul (2006) found that the main advantage of 2-D colour TDI over pulsed wave TDI and colour M-mode TDI was its ability to simultaneously quantify velocities in several segments within 1,2 or 3 myocardial walls in order to calculate different velocity gradients and to assess intra- and intraventricular synchrony, respectively.

2.10.4 Colour M-mode

With the colour M-mode, myocardial velocities were analysed along a selected single scan line which was placed in the same manner as for conventional
transventricular M-mode (Sahn et al., 1978) using a right parasternal longitudinal or circumferential ventricular view. Due to a high sampling rate, M-mode detected subtle changes in myocardial motion velocities and by comparison with 2D TDI imaging, M-mode TDI afforded a much higher temporal resolution and also greater signal-to-noise ratio (Garot et al., 1998). M-mode TDI tracings show on the same image both systolic and diastolic velocities within the entire wall thickness. Chetboul (2002) reported that as with the 2D colour TDI mode, myocardial velocities toward the transducer are encoded in red, and those away from the transducer in blue. Systole starts with the short isovolumic contraction phase, followed by the ejection phase with higher velocities. During the diastole, 4 phases might be identified: diastole starts with a short isovolumic relaxation phase followed by the early filling phase with much higher velocities. The third phase with lower velocities was diastasis, followed by the late filling phase with higher velocities (therefore brighter colours) during atrial contraction and off-line analysis using specific software allowed quantification of myocardial velocities in the different layers.

Schober et al. (2011) reported that resolution of CHF was associated with predictable changes in respiratory rate, serum NT-proBNP concentration, and selected tissue Doppler echocardiographic variables in dogs with DCM and MVD.

Choi et al. (2013) reported that the mean maximal systolic velocity was 6.92±1.78 cm/sec, the mean early diastolic velocity (Em) was 6.58±1.81 cm/sec, the mean late diastolic velocity (Am) was 5.10±2.00 cm/sec, the mean isovolumic contraction time (IVCT) was 53.61±95.13 msec, and the mean isovolumic relaxation time (IVRT) was 26.74 ± 57.24 msec. The early diastolic mitral inflow velocity (E)/Em ratio was 10.94 ± 3.27 while the Em/Am ratio was 1.40 ± 0.40. There was a negative correlation between Am duration and Am amplitude, and a positive correlation between the IVRT and Em/Am ratio (p < 0.05). The normal LV parameter using pulsed TDI method could be used as the reference range for identifying myocardial dysfunction in dogs.

Pseudonormalization
In pseudonormalization mitral valve profile may appear normal despite impaired diastolic function. As left atrial pressure increases with advancing diastolic dysfuction, early trasmitral flow velocity increases secondary to the increase in LA pressure. This changes the low transmitral valve E:A ratio back to normal (pseudonormal). As LA pressure increases even further, the restrictive filling pattern develops and E:A ratio becomes significantly greater than 1. During the time that LA pressures are just high enough to return the transmitral flow appearance to normal, IVRT also returns to normal (Nishimura\textit{et al.}, 1989; Nishimura\textit{b} \textit{et al.}, 1989; Fuentes, 2003)

2.11 CARDIAC BIOMARKER: PLASMA N-TERMInAL-PRO B-TYPE NATRIURETIC PEPTIDE (NT-proBNP) ASSAY

Plasma concentration of BNP and its precursors, N-terminal pro-BNP was found to be increased in patients with congestive heart failure and had been shown to accurately predict clinical severity and left ventricular ejection fraction (LVEF) as well as morbidity and mortality in those patients (Richards \textit{et al.}, 1998).

Sisson (1999) reported an increased concentration of BNP in early stage of heart disease in dogs.

Troughton \textit{et al.} (2000) reported that BNP-guided therapy was superior to conventional methods including echocardiography. Häggström \textit{et al.} (2000) also opined that measurement of BNP in plasma might be a useful tool for therapeutic decision.

McCullough (2002) stated that BNP measurement would add to clinical judgment in establishing a final diagnosis of congestive heart failure in human cardiac patients with acute dyspnoea.

Archer (2003) opined that ventricular, myocardial disease in dogs might be best detected and monitored by BNP and pro-BNP assays.

For a biomarker to be valuable in clinical practice, it needed to be measured rapidly and accurately at a reasonable cost, add diagnostic or prognostic information to available methods and help to guide patient management. BNP and N-terminal BNP fulfil most of those criteria in patients with suspected heart
failure and had been proven to be better than atrial natriuretic peptide (ANP) or N-terminal ANP in disease prediction and prognosis (Lemos et al., 2003).

In a study, MacDonald et al. (2003) found that plasma BNP was high in dogs with moderate to severe mitral valve disease and congestive heart failure. The authors also suggested that severe increase in plasma BNP might be of predictive value in premature death due to cardiovascular disease in the veterinary field, as in human medicine.

Nicholls et al. (2004) predicted that BNP and NT-BNP assays would gain popularity over ANP and NT-ANP because of its powerful statistical associations with hemodynamics, morbidity and mortality than ANP and NT-ANP in patients with disorders of the cardiovascular system.

Vanderheyden et al. (2004) reported that natriuretic peptides have emerged as important candidate for development of diagnostic tools and therapeutic agents in cardiovascular disease.

Steg et al. (2005) stated that BNP levels were elevated in patients with left ventricular dysfunction and found to be correlated with New York Heart Association functional classification as well as patient progress. Plasma concentration of BNP was found to be increased consistently with symptoms of heart failure and was regarded as an excellent hormonal marker for ventricular systemic dysfunction (Thomas et al., 2006). Boswood et al. (2007) claimed that NT-proBNP had a greater accuracy in distinguishing cardiac disease from respiratory diseases when compared with plasma pro ANP 31-67 concentration,

Despite fast advances in management and therapeutic aspects, patients with chronic congestive heart failure still reported to have an adverse prognosis over time. Since heart failure evoked the activation of multiple neurohormone, the use of natriuretic peptide for the confirmatory diagnosis and definitive prognosis was proven to be of growing interest (Miller et al., 2007).

In a retrospective study, Prosek et al. (2007) found that plasma BNP was significantly higher in patients with cardiac dyspnoea with 86.4 per cent sensitivity and 80.8 per cent specificity.
Oyama et al. (2009) stated that serum NT-proBNP cut-off concentration >1,158 pmol/L discriminated between dogs with congestive heart failure and dogs with primary respiratory tract disease with a sensitivity of 85.5 per cent and a specificity of 81.3 per cent.

Serres et al. (2009) found that in canine symptomatic MVD a threshold of 1500 pmol/L could discriminate survivor from non-survivor dogs with a sensitivity and specificity of 80 per cent and 73 per cent, respectively.

Schmidt et al. (2009) reported that dogs with renal disease had significantly higher mean serum concentration of NT-proBNP with normal cardiac function.

The diagnosis and management of canine heart disease could be facilitated by a highly sensitive and specific laboratory test that predicts risk of morbidity and mortality is helpful in directing therapy, easy to perform, inexpensive, and widely available. Veterinary cardiac biomarkers, specifically NT-proBNP, hold great promise (Oyama and Singletary, 2010).

Hori et al. (2010) found that Plasma ANP was a useful, non-invasive parameter for measuring rapid haemodynamic changes.

Moonarmart et al. (2010) reported that N-terminal pro B-type natriuretic peptide concentration and left ventricular end-diastolic diameter were significantly and independently predictive of all-cause mortality in dogs with degenerative mitral valve disease.

Kellihan et al. (2011) reported that NT-proBNP concentration was significantly higher in dogs with pre-capillary PH when compared to dogs with respiratory disease without PH, and NT-proBNP may be useful to predict the severity of estimated PH.

Ettinger et al., (2012) reported that a cut-off of 874 pmol/L, sensitivity and specificity of NT-proBNP concentration to detect clinical signs of cardiac disease were 70 per cent and 83 per cent, respectively; for VHS, sensitivity and specificity were 56 per cent and 85 per cent, respectively, at a cut-off of 11.5. Mean NT-proBNP concentration was significantly increased in dogs with cardiac-related
dyspnoea or coughing, compared with dogs in which these signs were noncardiac related.

Wolf et al., (2012) reported that dogs with follow-up NT-proBNP level <965 pmol/l had a significantly longer overall cardiac survival than patients with NT-proBNP level >965 pmol/l.

2.12 POST-MORTEM CHANGES
2.12.1 Gross pathology

Myocardial eccentric hypertrophy was evident by increased heart weight/body weight ratio, together with a decreased ratio of left ventricular wall thickness to chamber diameter. (Sisson and Thomas, 1995; Tidholm and Jonsson, 1996; Sisson et al., 1999).

Tidholm and Jonsson (1996) in the post-mortem examination with DCM, observed moderate to severe cardiomegaly. They also recorded pulmonary oedema in 95 per cent, hepatic congestion in 75 per cent, ascites in 40 per cent cases and pleural effusion in 30 per cent cases.

Gross pathological examination of heart in dogs with DCM, generally showed dilatation of either all four cardiac chambers or predominant dilatation of the left chambers (Tidholm and Jonsson, 1997; Sisson et al., 1999).

In valvular endocardiosis, affected valves were shortened and thick, either diffused or nodular and appeared smooth. Microscopically, the thickened valves had markedly increased fibroelastic proliferation and deposition of acid muco polysacharides. Myocardial alterations included arteriosclerosis of intra myocardial arteries and multifocal myocardial necrosis and fibrosis (Thomason et al., 2006).

Darke et al. (1996) studied gross changes in a dog with tricuspid dysplasia. There was severe right sided cardiomegaly and the right auricle was severely dilated owing to florid tricuspid regurgitation. The valve was abnormally formed with fused papillary muscles, abnormal leaflets and short chordae tendinae.

The gross pathological changes in dogs with combined subaortic stenosis and mitral valve dysplasia were thickened mitral leaflets with nodular surface,
short chordae tendinae, fibrous band at the entrance of the left ventricular outflow tract and above the septal leaflet of the mitral valve resembling scar tissue (Pikula et al., 2005).

Shigel et al. (2007) carried out histologic study of the cardiac conduction system in canine cases of mitral valve endocardiosis with complete atrioventricular block in which the upper portion of the lower bundle branch had been totally obliterated by the fibrotic process and the few remaining conduction fibers revealed granulation, vacuolation or lysis of the sarcoplasm with pyknotic or karyolytic nuclei.

2.12.2 Histopathology

The presence of attenuated wavy fibers was a major histological finding in dogs with DCM (Tilley and Liu, 1975; Tidholm et al., 1998 and Dambach et al., 1999). Three different reports on histological examinations of a total of 64 Doberman Pinschers with DCM described findings similar to cardiomyopathy of Boxers, i.e. fibrosis, fatty infiltration, myofibre degeneration, myocyte atrophy, and sometimes vacuolization (Calvert et al., 1982 and Everett et al., 1999).

In myocardial eccentric hypertrophy, the sarcomeres were increased in numbers in series rather than parallel, as in concentric hypertrophy. Myocardial eccentric hypertrophy was commonly caused by volume overload (Kittleson, 1994). Harpster (1983) described distinct histological characteristics in a group of Boxers, i.e. Boxer cardiomyopathy. However, no major breed specific differences concerning clinical, pathological, or prognostic characteristics were found in dogs with DCM (Tidholm and Jonsson, 1997; Tidholm et al., 1997).

Tidholm et al. (2001) classified canine idiopathic Dilated Cardiomyopathy into fatty infiltration-degenerative type and attenuated wavy fibre type and seemed superior to classification suggesting, breed-specific syndromes, as some breeds (i.e. Boxers and Doberman pinchers) could be affected by both diseases.

Tidholm and Jonsson (2005) stated that, histological findings in dogs with clinically diagnosed DCM revealed two distinct forms of DCM the first one is cardiomyopathy of Boxers and Doberman Pinschers, corresponding to the “fatty infiltration–degenerative” type and second form seen in many giant, large-,
medium-sized breeds, including some Boxers and Doberman Pinschers, classified as the ‘‘attenuated wavy fibre’’ type of DCM.
CHAPTER III
MATERIALS AND METHODS

The study entitled “Doppler echocardiographic evaluation of acquired heart diseases in dogs” was carried out at the Centre for Advanced Faculty Training in Veterinary Clinical Medicine, Ethics and Jurisprudence, Madras Veterinary College, Chennai-600007 for a period of five semesters from February, 2012 to February, 2014.

3.1 PATIENT SELECTION

In the study period the number of dogs brought to Madras Veterinary College Teaching Hospital was 28469. Out of which 234 dogs were with signs of cardiac signs. In which 106 cases were diagnosed as acquired heart diseases with cardiac failure based on echocardiography and they were selected for this study.

Apparently healthy animals

Twenty apparently healthy dogs brought for routine check-up and vaccination were randomly selected to collect reference values of the parameters under study.

Clinical cases

The sick dogs brought to Small Animal Clinic, Outpatient Medical Unit of Madras Veterinary College Teaching Hospital, with clinical signs suggestive of cardiac failure were screened. They were subjected to physical examination, Haemato-biochemical and special examinations including radiography, electrocardiography, systolic blood pressure measurement, echocardiography, Tissue Doppler Imaging, Post-mortem examination and NT-proBNP marker assay.

3.2 DESIGN OF THE STUDY

The study consisted of five groups which included apparently healthy dogs and clinical cases of acquired heart diseases with heart failure.

Groups of Clinical Study

Group I: Apparently healthy dogs (Control group) (n=20)

Group II: Dilated Cardiomyopathy (DCM) (n=58)
Group III: Mitral Valve Disease (MVD) (n=39)
Group IV: Pericardial effusion (n=6)
Group V: Hypertrophic Cardiomyopathy (HCM) (n=3)

3.3 CLINICAL STUDY

All the selected animals were subjected to routine clinical examination comprising of physical examination as suggested by McCurin and Poffenbarger (1991) and detailed cardiovascular assessment as suggested by Tilley et al. (1992) and Ware (2007) and Ettinger and Feldman (2010) (Appendix-I). All the selected cases were subjected to routine laboratory investigations as per standard clinical laboratory protocols suggested by Barger (2003) and Gunn and Alleman (2005) as per standard clinical laboratory protocols.

3.4 PARAMETERS OF THE STUDY

The following clinical and laboratory parameters were studied in apparently healthy dogs and in the clinical cases of acquired heart diseases (Appendix-I).

3.4.1 Prevalence

The data on breed, age, sex were collected for demographic studies during the study period of five semesters.

3.4.2 Clinical Presentation

Chief complaints, age at onset, management practices, medication history and chronology of events were assessed. Appetite, physical activity, dyspnoea, abdominal enlargement, pulsation and additional clinical findings were assessed.

Lethargy, weakness, exercise intolerance, syncope, weak femoral pulse with pulse deficit, tachycardia, concurrent progressive or refractory congestive heart failure were assessed through clinical examination.

3.4.3 Baseline Haematology Panel

3.4.3.1 Erythrogram:

Haemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), White Blood Cell (WBC) count and Platelet count were estimated.

3.4.3.2 Leucogram:
Total Leucocyte Count (TLC) and Differential Leucocyte Count (DLC) were estimated.

3.4.4 Baseline Serum Biochemistry Panel

Blood Urea Nitrogen (BUN), Creatinine, Alanine transaminase (ALT), Alkaline Phosphatase (ALP), Total protein, Albumin, Calcium, Phosphorous, Sodium and Potassium levels in the serum were estimated.

3.4.5 Radiography

Cardiac size and concurrent radiographic signs in the thoracic radiographs were studied.

3.4.6 Doppler Blood Pressure

Non-invasive systolic blood pressure measurements were recorded.

3.4.7 Electrocardiography

Baseline ECG was recorded in selected animals and the values were obtained. Rhythm and complex morphology was analysed for categorization of arrhythmias.

3.4.8 Echocardiography

a) Two-dimensional echocardiographic indices
   1. Left Atrial diameter in cm (LA)
   2. Aortic diameter in cm (Ao)
   3. LA/Ao ratio

b) M-mode echocardiographic indices
   1. Right Ventricular Internal Diameter during Diastole (RVIDd cm)
   2. Right Ventricular Internal Diameter during Systole (RVIDs cm)
   3. Inter Ventricular Septum during Diastole (IVSd cm)
   4. Inter Ventricular Septum during Systole (IVSs cm)
   5. Left Ventricular Internal Diameter during Diastole (LVIDd cm)
   6. Left Ventricular Internal Diameter during systole (LVIDs cm)
   7. Left Ventricular Posterior Wall during Diastole (LVPWd cm)
   8. Left Ventricular Posterior Wall during Systole (LVPWs cm)
   9. Fractional Shortening (FS %)
10. E-Point to Septal Separation (EPSS cm)

c) Colour flow Doppler
   1. Mitral Valve flow
   2. Tricuspid Valve flow
   3. Aortic flow
   4. Pulmonary Artery flow

3.4.9 Tissue Doppler Imaging (TDI)
   1. Pulsed-Wave TDI indices
      1. Peak Systolic Velocity (Sm) (m/sec)
      2. Early Diastolic Velocity (Em) (m/sec)
      3. Late Diastolic Velocity (Am) (m/sec)
      4. Em/Am ratio
      5. Isovolumic Relaxation Time (IVRT) (ms)
      6. Isovolumic Contraction Time (IVCT) (ms)
   2. 2-Dimensional TDI
   3. Colour M-mode TDI

3.4.10 Cardiac Biomarker: NT-proBNP Assay

3.4.11 Post-mortem examination

3.5 COLLECTION AND EXAMINATION OF CLINICAL MATERIALS

3.5.1 Collection of Blood Samples

   Approximately 10 ml of blood was collected from either cephalic vein or recurrent tarsal vein as per standard protocols, for haemato-biochemical studies. 2.0 ml of blood was transferred into a dry vial containing 10 per cent EDTA for complete haematological studies. 5.0 ml of blood was transferred into serum tubes taking all precautions for avoiding haemolysis, as suggested by Meinkoth and Clinkenbeard (2002) for harvesting serum for the purpose of biochemical studies and NT-proBNP estimation.

3.6 EXAMINATION OF CLINICAL MATERIALS

3.6.1 Examination of Blood Samples
Haematological analysis was done using automated haematology analyser (Mindray-BC-2800 Vet\(^1\)) and parameters such as Haemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), Platelet count, Leucocyte count and Differential count were analysed, following manufacturer’s recommendations for respective estimations (Plate-I).

3.6.2 Examination of Biochemical samples

Biochemical estimations were carried out using fully automated biochemical analyser (A-15 Biosystem Random Access Analyzer\(^2\)) (Plate-I). Quantitative estimation of Blood Urea Nitrogen (BUN), Creatinine, Alanine Transferase (ALT), Alkaline Phosphatase (ALP), Total protein, Albumin, Sodium, Potassium, Calcium and Phosphorous were carried out using specific diagnostic kits supplied by Agappe Diagnostics Private Limited, India, following the manufacturer’s recommendations for respective estimations.

3.7 SPECIAL DIAGNOSTIC PROCEDURES FOR CARDIOVASCULAR ASSESSMENT

3.7.1 Doppler Blood Pressure

The Instrument used for the present study was Vmed Vet-Dop\(^3\)(Plate-I). It consisted of Doppler detector, sphygmomanometer, ultra sound gel and different sized cuffs. Animal was placed on right lateral recumbency, either forelimb or hind limb was used, limb circumference at the site of cuff placement was measured using an inch tape. The cuff whose width was about 40 per cent of the limb circumference was used for blood pressure measurement. The cuff was consistently placed in the left forearm region at the level of heart and about 10cm from the heart (i.e. over the mid – ante brachium). As the superficial palmar arterial arch is used for blood pressure measurement, the hair over the area distal to palmar meta carpel pad was clipped and the appropriate sized cuff was positioned over the mid- ante brachium. Ultrasound gel was applied to get good contact between the skin and the transducer. The cuff is attached to a pressure

\(^1\)Auto Hematology Analyzer, Mindray medical India Pvt Ltd, India
\(^2\)A15 Random Access Analyzer, Biosystems, Barcelona, Spain
\(^3\)Ultrasonic Doppler Animal Blood Pressure System, Vmed Technology
manometer and inflated and deflated manually. The first sound heard as the cuff gradually deflates is recorded as the systolic pressure. The diastolic pressure is estimated by a change in the character of the pulsing sound as the cuff deflates. Five subsequent measurements were taken and the average value was taken for analysis. Systolic blood pressure was measured using Doppler BP apparatus as per standard protocols of Carr (2001).

3.7.2 Radiographic examination

All the animals under the study were subjected to radiographic examination as per standard techniques suggested by Buchanan (2000). Based on the Vertebral Heart Score (VHS), the cardiac size of the selected clinical cases was assessed and cardiomegaly was evaluated. Cardiac and Pulmonary structures were assessed and interpreted for cardiovascular disorders following standard protocols of Buchanan and Bucheler (1995) and Ettinger and Feldman (2010).

3.7.3 Electrocardiogram

Selected healthy dogs and clinical cases were subjected to routine ECG. Welch Allyn CP 50™ ECG unit (Plate-I) was used to obtain baseline ECG. The animal was placed on right lateral recumbency and the ECGs were obtained with standard bipolar limb lead system as described by Tilley (1992) and Ware (2007). ECG was interpreted as per the standard interpretation protocols of Tilley (1992).

3.8 ECHOCARDIOGRAPHY

Healthy dogs and selected clinical cases were subjected to echocardiographic examination using ALOKA ProSound 3500SX system (Plate-II) (Aloka India Ltd.). 2D, M-Mode, Doppler Echocardiographic assessments and Tissue Doppler Imaging were carried out as per standard protocols of Boon et al. (1983) and Miller et al. (1989). The echocardiographic parameters were interpreted as per Bonagura (1983) and Thomas et al. (1993).

The Echocardiographic indices included in this study are Left ventricular Fractional Shortening (FS), E Point to Septal Separation (EPSS), Ejection Fraction

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4WelchAllyn CP 50™ Electrocardiograph, Welch Allyn Inc, USA
5Hitachi Aloka Medical Ltd
(EF), Left Ventricular Internal Diameter during Diastole (LVIDd), Left Ventricular Internal Diameter during systole (LVIDs), Right Ventricular Internal Diameter during Diastole (RVIDd), Right Ventricular Internal Diameter during Systole (RVIDs), Inter Ventricular Septum during Diastole (IVSd), Inter Ventricular Septum during Systole (IVSs), Left Ventricular Posterior Wall during Diastole (LVPWd) and Left Ventricular Posterior Wall during Systole (LVPWs).

### 3.8.1 Preparation and positioning of the animal

Suspected cases after physical examination were kept in a calm place to minimize the stress. Fifteen minutes late hair coat was clipped between the costochondral junction and the sternum on both sides of the thorax in the area of the right and left parasternal windows and all the echocardiographic measurements were carried out with the animal lying in lateral recumbency. Each animal was positioned in right and left lateral recumbency, and imaged from below through a hallow window in the table (Koch et al., 1996).

### 3.8.2 Two dimensional echocardiography

It was done at right parasternal short axis view which was obtained at the fourth and fifth intercostal spaces ventrally between the sternum and the costochondral junction. From the long-axis views, the index marker of the transducer was directed towards the head of the patient to obtain short-axis views.

Two dimensional measurements for Ao and LA were obtained from a short-axis plane at the level of the aortic valves. The first frame after the aortic excursion was used, which is early diastole. In 2-D, transverse dimensions of Ao and LA were measured. For Ao, the first calliper was placed at the midpoint of the convex curvature of the wall of the right aortic sinus. The second calliper was positioned at the point where the aortic wall and the non-coronary and the left coronary aortic cusps merge. The LA was measured by extending the line from the same point where the second calliper was positioned to the blood-tissue interface of the LA wall. The LA/Ao ratios were calculated as an index for left atrial size (Boon, 2011) (Plate-VI).

### 3.8.2.1 Simpson’s method of Disc
This method was used to measure the chamber volumes in Group II with DCM only. Left apical four chamber view was used. It was obtained by placing the transducer over the apex, the ultrasound beam was directed almost perpendicular to the sternum and parallel to the long axis of the heart with reference mark directed toward the spine and the crystals pointing towards the neck (cable to knees), about 30 degrees between chest wall and transducer. By rotating the ultrasound beam in a left caudal to right cranial direction and angling dorsally towards the base of the heart, a four-chamber view was obtained showing the left ventricle and atrium on the right side and the right ventricle and atrium on the left side. (Boon, 2011).

SMOD measurements were done on left apical four chamber view with selection of end diastolic frames (at the time of mitral valve closure) and end-systolic frames (corresponding to the last frame before mitral valve opening). The LV area was measured by tracing the endocardial border on each selected image; maximal LV length was measured from the middle of a line connecting the two mitral annuli to the endocardial border of the LV apex. LV volumes were then automatically calculated by the ultrasound machine. Ejection Fraction was calculated from SMOD derived end-diastolic and end-systolic LV volumes by using the following formula:

\[
\text{Ejection Fraction} = \frac{EDV - ESV}{EDV} \times 100 \text{ per cent} \quad (\text{Boon, 2011})
\]

3.8.3 M-mode echocardiography

Left ventricle M-modes were obtained in right parasternal long axis and short axis views. In right parasternal long-axis LV outflow view the cursor was positioned perpendicular to the IVS and LV free wall at the level of chordae tendinae, between the mitral valve leaflets and the LV papillary muscles (Boon, 2011).

M-mode right parasternal transverse images were obtained from the real time by placing the cursor over the septum and free wall, bisecting the image into perfect right and left halves. Smallest circular left ventricle at the level of the chordae on transverse image was used for M-mode images. The M-mode image
had RV at the top of the image, followed by the IVS, the LV chamber, and then LV free wall at the bottom of the image (Plate-VI).

For mitral valve EPSS, M-mode right parasternal transverse mitral valve view was used. This view called as fish mouth view. The cursor was placed perpendicular to the valves as it divides the image into equal and similar halves. In this view the shortest distance from the E point (EPSS) of the mitral valve to the ventricular septum was measured (Boon, 2011).

The resulting M-mode imaging as depth through the heart on the Y-axis and time on the X-axis and the following measurements were collected: Left ventricular diameter at end diastole (LVIDd), left ventricular diameter at end-systole (LVIDs), left ventricular posterior wall thickness at end-diastole (LVPWd), left ventricular posterior wall thickness at end-systole (LVPWs), interventricular septal thickness at end-diastole (IVSd) and interventricular septal thickness at end-systole (IVSs). From these above values the contraction indices like fractional shortening, interventricular septum fraction thickening and left ventricular posterior wall fraction thickening were calculated using the following formulas.

\[
\text{Fractional shortening} = \frac{\text{LVIDd} - \text{LVID}}{\text{LVIDd}} \times 100 \text{ per cent (Boon, 2011)}
\]

\[
\text{Interventricular septum fraction thickening} = \frac{\text{IVSs} - \text{IVSd}}{\text{IVSd}} \times 100 \text{ per cent (Boon, 2011)}
\]

\[
\text{Left ventricular posterior wall fraction thickening} = \frac{\text{LVPWs} - \text{LVPWd}}{\text{LVPWd}} \times 100 \text{ per cent (Boon, 2011)}
\]

3.8.4 Colour Flow Doppler Echocardiography

In colour flow Doppler, the flow of blood was portrayed on a 2D echocardiographic image of the heart, as a form of angiogram. Laminar blood flow towards the transducer was red; flow away from the transducer was blue. As blood flows at lower velocities, the colour became dark. High flow velocities as seen in regurgitations led to aliasing and a colour shift. Turbulences appeared in yellow or green colours (Darke et al., 1993) (Plate-VI).

3.9 TISSUE DOPPLER IMAGING (TDI)

In Tissue Doppler Imaging (TDI) low frequency high amplitude sound was used to record myocardial velocity during systole and diastole. It was a reverse
Doppler. 2D colour TDI and Colour M-mode TDI was performed in two dimensional mode by superimposing colour flow or M-mode.

3.9.1 Pulsed-wave TDI (Boon, 2011)

Pulsed-wave TDI was obtained in real time by placing a gate over a portion of myocardium and recording positive and negative frequency shifts. Pulsed-wave TDI provided information from one gate location in real time, and the gate was moved to another location to interrogate any other area of the myocardium. Mitral annulus velocity was measured in all cases as standard as it represents the regional and global velocity of myocardium. (Plate-VII-c)

Pulsed-wave TDI was used to measure peak myocardial velocities especially in the measurement of long-axis ventricular motion because the longitudinally oriented endocardial fibers are most parallel to the ultrasound beam in the apical views. Because the apex remains relatively stationary throughout the cardiac cycle, mitral annular motion was a good surrogate measure of overall longitudinal left ventricular (LV) contraction and relaxation (Vinereanu et al., 1999). Mitral annulus velocity determined by TDI was a relatively preload-independent variable in evaluating diastolic function (Sohn et al., 1997).

3.9.1.1 Imaging planes

PW TDI longitudinal myocardial velocity was obtained from apical four-chamber views of the heart at mitral annulus. The lateral walls of the left and right ventricular chamber or the interventricular septum are positioned on the image so that the colour sector or Doppler cursor line up parallel with the length of the wall or septum. PW TDI was obtained immediately by placing along the wall or septum.

3.9.1.2 Spectral Appearance

In the PW TDI image, the baseline placed in the middle of the spectral display. Systolic contraction (Sm) resulted in a positive frequency shift. Diastolic motion of the myocardium was negative and has two phases: Early diastolic motion (Em) and late diastolic motion (Am) secondary to the atrial contraction. Between the diastolic and systolic waves, isovolumic contraction and relaxation
times were identified. Superimposed Em and Am were recorded in cases with faster heart rates.

3.9.2 2D Colour TDI

The 2D color TDI mode gave the best “overall impression” of myocardial movements qualitatively because all velocity data were displayed simultaneously on the screen. The right parasternal short axis view and the left parasternal long axis view (4 or 5 chambers) allowed the qualitative analysis of the transverse and longitudinal myocardial motions, respectively (Chetboul, 2002) (Plate-VII-a).

3.9.3 Colour M-mode

With the color M-mode, myocardial velocities were analysed qualitatively along a selected single scan line which was placed in the same manner as for conventional transventricular M-mode, used in right parasternal longitudinal or circumferential ventricular view (Chetboul, 2002) (Plate-VII-b).

3.9.4 Global TDI Velocity

Global TDI velocity is the velocity of whole left ventricle during systole and diastole, and it was calculated from the Sm, Em and Am velocities.

\[
\text{Global TDI} = 5m \times \frac{Em}{Am}
\]

3.10 CARDIAC BIOMARKER: NT-proBNP ASSAY

The animals which showed signs suggestive of cardiac disease were selected for this parameter especially with dyspnoea and exercise intolerance. For this study 34 dogs were randomly selected out of 234 dogs screened. Six healthy controls were selected from the twenty healthy controls. Based on the assay results and echocardiography results the animals were grouped as control, DCM, MVD with systolic failure, MVD without systolic failure, occult cardiac disease and others. In all 34 cases echocardiography was done to compare the efficacy of the marker (Plate-VIII).

3.10.1 Sampling

Blood was collected by venepuncture into prechilled EDTA vaccutainer tube of 5 ml capacity and was centrifuged within one hour of collection at 2,000 RPM at 4°C for 15 minutes. The plasma was separated and frozen immediately at
-20°C and stored until the assay was carried out. From plasma samples, NT-pro BNP was estimated by using the CUSABIO Canine NT-proBNP ELISA kit.

3.10.2 Principle of the Assay

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for NT-proBNP had been pre-coated onto a microplate. Standards and samples were pipetted into the wells and any NT-proBNP present was bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for NT-proBNP was added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) was added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution was added to the wells and colour develops in proportion to the amount of NT-proBNP bound in the initial step. The colour development was stopped and the intensity of the colour is measured.

3.10.3 Sensitivity and Specificity

The detection range was from 0.312 ng/ml to 20 ng/ml. The minimum detectable dose of canine NT-proBNP was typically less than 0.078 ng/ml. The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. It was determined the mean O.D value of 20 replicates of the zero standard added by their three standard deviations. This assay had high sensitivity and excellent specificity for detection of canine NT-proBNP. No significant cross-reactivity or interference between canine NT-proBNP and analogues was observed.

3.10.4 Materials Required

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay plate (12x 8 coated Microwells)</td>
<td>1(96 wells)</td>
</tr>
<tr>
<td>Standard (Freeze dried)</td>
<td>2</td>
</tr>
<tr>
<td>Biotin-antibody (100 x concentrate)</td>
<td>1 x 120 μl</td>
</tr>
<tr>
<td>HRP-avidin (100 x concentrate)</td>
<td>1 x 120 μl</td>
</tr>
<tr>
<td>Biotin-antibody Diluent</td>
<td>1 x 15 ml</td>
</tr>
<tr>
<td>HRP-avidin Diluent</td>
<td>1 x 15 ml</td>
</tr>
<tr>
<td>Sample Diluent</td>
<td>1 x 50 ml</td>
</tr>
</tbody>
</table>
Wash Buffer (25 x concentrate)  1 x 20 ml
TMB Substrate  1 x 10 ml
Stop Solution  1 x 10 ml
Adhesive Strip (For 96 wells)  4

3.10.5 Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- An incubator with stable incubation conditions up to 37°C±0.5°C.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Absorbent paper for blotting the microtiter plate.
- 100 mL and 500 mL graduated cylinders.
- Deionized or distilled water.
- Pipettes and pipette tips.
- Test tubes for dilution.

3.10.6 Sample Collection and Storage

Used a serum separator tube (SST) and allowed samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1000 ×g. Removed serum and assayed immediately or aliquot and stored samples at -20°C or -80°C. Repeated freeze-thaw cycles was avoided.

3.10.7 Reagent Preparation

- All reagents in room temperature (18-25°C) before use for 30min.
- Prepared fresh standard for each assay. Used within 4 hours and discarded after use.
- No serial dilution directly in the wells was carried out.
- Carefully reconstituted Standards according to the instruction, and avoided foaming and mixed gently until the crystals have completely dissolved. To minimize imprecision caused by pipetting, used small volumes and ensured that pipettes are calibrated. It is recommended to suck more than 10μl for once pipetting.
- Distilled water was used to make the preparation for reagents or samples.

1. **Biotin-antibody (1x)**
   
The vial centrifuged before opening. 100-fold dilution was prepared by adding 10 μl of Biotin-antibody in 990 μl of Biotin-antibody Diluent.

2. **HRP-avidin (1x)**
   
The vial centrifuged before opening. 100-fold dilution was prepared by adding 10 μl of HRP-avidin in 990 μl of HRP-avidin Diluent.

3. **Wash Buffer (1x)**
   
   If crystals have formed in the concentrate then it was warmed up to room temperature and mixed gently until the crystals had completely dissolved. 20 ml of Wash Buffer Concentrate (25 x) was diluted with deionized or distilled water to prepare 500 ml of Wash Buffer (1 x).

4. **Standard**
   
The standard vial was centrifuged at 6000-10000 rpm for 30s and reconstituted the Standard with 1.0 ml of Sample Diluent. This reconstitution produced a stock solution of 20 ng/ml. The standard was mixed to ensure complete reconstitution and allowed the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

   250 μl of Sample Diluent was pipetted into each tube (S0-S6). The stock solution was used to produce a 2-fold dilution series (as below). Each tube was mixed thoroughly before the next transfer. The undiluted Standard served as the high standard (20 ng/ml). Sample Diluent served as the zero standard (0 ng/ml).

<table>
<thead>
<tr>
<th>Tube</th>
<th>S7</th>
<th>S6</th>
<th>S5</th>
<th>S4</th>
<th>S3</th>
<th>S2</th>
<th>S1</th>
<th>S0</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng/ml</td>
<td>20</td>
<td>10</td>
<td>5</td>
<td>2.5</td>
<td>1.25</td>
<td>0.625</td>
<td>0.312</td>
<td>0</td>
</tr>
</tbody>
</table>
3.10.8 Assay Procedure

All reagents and samples were kept in room temperature before use. Centrifuged the sample again after thawing before the assay. All 40 samples and 8 standards were assayed in duplicate.

1. All reagents, working standards, and samples were prepared as directed in the previous sections.

2. 100μl of standard and sample were added per well. Covered with the adhesive strip provided. Incubated for 2 hours at 37°C. A plate layout was used to record standards and samples assayed.

3. The liquid of each well was removed.

4. 100μl of Biotin-antibody (1x) was added to each well. Covered with a new adhesive strip. Incubated for 1 hour at 37°C.

5. Each well was aspirated and washed and repeated the process two times for a total of three washes. Washed by filling each well with Wash Buffer (200μl) using multi-channel pipette and let it stand for 2 minutes. The liquid completely removed at each step, because it was essential for good performance. After the last wash, remaining wash buffer was removed by aspirating or decanting. The plate was inverted and blotted against clean paper towels.

6. 100μl of HRP-avidin (1x) was added to each well. The microtiter plate was covered with a new adhesive strip. Incubated for 1 hour at 37°C.

7. The aspiration/wash process was repeated for five times as in step 5.

8. 90μl of TMB Substrate was added to each well. Incubated for 15-30 minutes at 37°C and protected from light.

9. 50μl of Stop Solution was added to each well, the plate was gently tapped to ensure thorough mixing.

10. The optical density of each well determined within 5 minutes, using a microplate reader\(^6\) set to 450 nm (Plate-VIII). One more reading was taken at

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\(^6\)Multiskan™ GO Microplate Spectrophotometer, ThermoScientific
540 nm or 570 nm for wavelength correction. Readings were subtracted at 540 nm or 570 nm from the readings at 450 nm. This subtraction was to correct the optical imperfections in the plate.

### 3.10.9 Calculation of Results

With use of professional soft “Curve Expert 1.4” or “MS Office Excel 2013” a standard curve was created.

Averaged the duplicate readings for each standard and sample and subtracted the average zero standard optical density.

A standard curve was created by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draws a best fit curve through the points on the graph. The data may be linearized by plotting the log of the NT-proBNP concentrations versus the log of the O.D. and the best fit line was determined by regression analysis. In diluted samples, the concentration read from the standard curve was multiplied by the dilution factor. A standard curve was plotted from the values measured and the concentration of NT-proBNP in the samples was calculated from the above.

### 3.11 POST-MORTEM EXAMINATION

Post-mortem examination was performed only in 4 dogs with DCM during the study period and the gross appearance of apex, papillary muscles, wall thickness and shape of heart chambers were examined (Plate-IX-a) and recorded.

The histopathological examination was performed by fixing the pieces of myocardial tissue in 10 per cent neutral buffered formalin processed through alcohol and xylene and then embedded in paraffin; sections were cut at 4-5 µm thickness and stained by haematoxylin and eosin and special stain (Picro Sirius Red staining) for differentiating the collagen fibres from myocytes (Plate-IX-b).

### 3.12 STATISTICAL ANALYSIS

The data obtained were subjected to statistical analysis as per Snedecor and Cochran (1994). The statistical software package – IBM SPSS Statistics v20 for Windows were utilized in this study and the results were analysed using one way ANOVA and post-hoc Duncan’s technique for comparing within groups and
post-hoc Dunnett’s technique for comparing the disease groups with healthy control group. The results having same superscript of control in a row do not differ significantly from control. The results were presented in figures, tables and graphs and were discussed critically.
CHAPTER IV
RESULTS

The study entitled “Doppler echocardiographic evaluation of acquired heart diseases in dogs” was carried out at the Centre for Advanced Faculty Training in Veterinary Clinical Medicine, Ethics and Jurisprudence, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai-7 during the period February, 2012 to February, 2014. The study was conducted in healthy and clinical cases of dogs with AHDs presented to the Small Animal Medicine Outpatient Clinic of the Madras Veterinary College Teaching Hospital.

4.1 DEMOGRAPHY

The prevalence of AHDs is presented in Fig-I and II. During the study period of two year, 28467 cases brought, 234 cases were suspected for cardiovascular diseases, and only 106 dogs out of this (28467) had AHDs. This formed an overall prevalence of 0.37 per cent. In this disease population the DCM was 54.72 per cent (58/106), MVD was 36.79 per cent, pericardial effusion was 5.66 per cent (6/106) and HCM was 2.83 per cent (3/106).

The breed wise incidence of AHDs are presented in Fig-III-a, III-b and III-c. Affected dogs in the present study included small, medium and large breeds of dogs. The most commonly affected breeds in Group II with DCM were Labrador retriever – 52 per cent (30/58), Doberman – 19 per cent (11/58) and Boxer – 7 per cent (4/58). In Group III with MVD the affected breeds were Spitz – 44 per cent (14/39); in Group IV with pericardial effusion ND – 67 per cent (4/6) were the commonly affected breed. In Group V with HCM Non-descript was the commonly affected breed – 67 per cent (2/3).

The age wise prevalence is presented in Fig-IV. The incidence was found to be higher in 8 – 10 years of age group. The per centage of prevalence was as follows <2 years -7.55 per cent (8/106), 2 - 4 years-13.21 per cent (14/106), 4 - 6 years-18.87 per cent (20/106), 6 -8 years-19.81 per cent (21/106), 8 -10 years-23.58 per cent (25/106), 10 - 12 years-10.38 per cent (11/106), and > 12 years-6.60 per cent (7/106).
Figure-I
Prevalence of AHD in overall population

Figure-II
Diseases wise prevalence
Figure-III-a
Breedwise incidence of DCM

- Labrador: 52%
- Doberman: 19%
- ND: 5%
- Boxer: 7%
- German Shepherd: 4%
- Great Dane: 3%
- Dalmatian: 2%
- Rottweiler: 3%

Figure- III-b
Breedwise incidence of MVD

- Labrador: 36%
- Spitz: 6%
- Doberman: 8%
- ND: 8%
- Boxer: 5%
- German Shepherd: 5%
- Great Dane: 2%
- Dalmatian: 2%
- Rottweiler: 2%

Figure- III-c
Breedwise incidence of pericardial effusion

- Labrador: 67%
- ND: 17%
- German Shepherd: 16%
The sex wise prevalence is presented in Fig-V. Male dogs were found to be dominating the prevalence (75 per cent) than female (25 per cent) during the study period.

4.2 MEDICAL HISTORY

The nature of presenting complaints is presented in Table-I. The complaints such as inappetance, exercise intolerance, abdominal enlargement and syncope were reported in varying degrees in different groups of AHDs. Abdominal enlargement was reported in high numbers (86.21 per cent) in Group II with DCM, which was followed by inappetance (72.41 per cent), exercise intolerance (68.97 per cent), weakness (62.07 per cent), dyspnoea (43.10 per cent) and syncope (10.34 per cent). Exercise intolerance (51.28 per cent) was reported in high numbers in Group III with MVD, which was followed by weakness (46.15 per cent), inappetance (30.77 per cent), dyspnoea (25.64 per cent), abdominal enlargement (12.82 per cent) and syncope (5.13 per cent). The other complaints were reported in less per cent in Group IV and Group V (Table-I). Syncope was the major sign reported in Group V with HCM.

4.3 CLINICAL PRESENTATION

The clinical findings in different groups of AHDs are presented in Table-II. Findings such as ascites, cough, pedal oedema, tachycardia, pulse deficit, murmur, pulmonary oedema, pale mucous membrane, vomiting, weight loss and melena were observed in varying degrees in different groups of AHDs.

Tachycardia was observed in almost equal levels in all the groups of dogs. Besides tachycardia the other predominant signs in Group II with DCM were murmur (89.66 per cent), ascites (82.76 per cent), pedal oedema (77.59 per cent) and pulmonary oedema (60.34 per cent). In Group III with MVD the major signs were coughing (89.74 per cent) and murmur (89.74 per cent) with minor signs of pulmonary oedema (38.46 per cent) and pedal oedema (20.51 per cent). Pulse deficit (83.33 per cent), ascites (66.67 per cent) and coughing (50 per cent) were common signs with in Group IV with Pericardial Effusion (Plate-III).
Figure-IV
Age wise prevalence

No. of animals

Age of animals

<2 years  2 - 4 years  4 - 6 years  6 - 8 years  8 - 10 years  10 - 12 years  > 12 years

8  14  20  21  25  11  7

Figure-V
Sex wise prevalence

Male
Female

25%
75%
Table-I
Medical History in Dogs with Acquired Heart Diseases (AHD)

<table>
<thead>
<tr>
<th>Chief Complaints</th>
<th>Group – II Dilated Cardiomyopathy (n=58)</th>
<th>Group – III Mitral Valve Disease (n=39)</th>
<th>Group – IV Pericardial Effusion (n=6)</th>
<th>Group – V Hypertrophic Cardiomyopathy (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inappetance (%)</td>
<td>72.41</td>
<td>30.77</td>
<td>33.33</td>
<td>66.67</td>
</tr>
<tr>
<td>Exercise intolerance (%)</td>
<td>68.97</td>
<td>51.28</td>
<td>33.33</td>
<td>66.67</td>
</tr>
<tr>
<td>Dyspnoea (%)</td>
<td>43.10</td>
<td>25.64</td>
<td>16.67</td>
<td>66.67</td>
</tr>
<tr>
<td>Abdominal enlargement (%)</td>
<td>86.21</td>
<td>12.82</td>
<td>83.33</td>
<td>-</td>
</tr>
<tr>
<td>Weakness (%)</td>
<td>62.07</td>
<td>46.15</td>
<td>66.67</td>
<td>66.67</td>
</tr>
<tr>
<td>Syncope (%)</td>
<td>10.34</td>
<td>5.13</td>
<td>-</td>
<td>100.00</td>
</tr>
</tbody>
</table>
Table-II
Clinical Presentation in Dogs with Acquired Heart Diseases (AHD)

<table>
<thead>
<tr>
<th>Chief Complaints</th>
<th>Group - II Dilated Cardiomyopathy (n=58)</th>
<th>Group – III Mitral Valve Disease (n=39)</th>
<th>Group – IV Pericardial Effusion (n=6)</th>
<th>Group – V Hypertrophic Cardiomyopathy (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tachycardia (%)</td>
<td>86.21</td>
<td>66.67</td>
<td>83.33</td>
<td>100.00</td>
</tr>
<tr>
<td>Murmur (%)</td>
<td>89.66</td>
<td>89.74</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pulmonary oedema (%)</td>
<td>60.34</td>
<td>38.46</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pulse deficit (%)</td>
<td>36.21</td>
<td>5.13</td>
<td>83.33</td>
<td>-</td>
</tr>
<tr>
<td>Ascites (%)</td>
<td>82.76</td>
<td>15.38</td>
<td>66.67</td>
<td>-</td>
</tr>
<tr>
<td>Coughing (%)</td>
<td>20.69</td>
<td>89.74</td>
<td>50.00</td>
<td>-</td>
</tr>
<tr>
<td>Pedal oedema (%)</td>
<td>77.59</td>
<td>20.51</td>
<td>33.33</td>
<td>-</td>
</tr>
<tr>
<td>Vomiting (%)</td>
<td>5.17</td>
<td>5.13</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Weight loss (%)</td>
<td>17.24</td>
<td>20.51</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Findings such as vomiting, weight loss and murmur were observed in some dogs in Group II and III. These signs were not observed in Group IV and Group V

4.4 HAEMOGRAM

The Erythrogram and leucogram values are presented in Table-III. The mean ± SE values of erythrogram of control group animals in this study were Haemoglobin -12.84±1.46 g/dl, Packed Cell Volume – 40.92±4.44 per cent and RBC count –5.49±0.42x 10^6/mm^3.

The mean ± SE values of leucogram, platelets, neutrophils, lymphocytes, monocytes and eosinophil counts of control group animals in this study were 12.76±1.60 x 10^3 cells/cmm, 2.47±0.61 lakhs/cmm, 73.00±2.58 per cent, 18.00±2.37 per cent, 3.25±0.70 per cent, 3.08±0.36 per cent respectively.

The mean haemoglobin, packed cell volume, red blood cell count, white blood cell count, platelets, neutrophils, lymphocytes, monocytes and eosinophil counts in Group I animals with DCM were 12.41±0.37 g/dl, 40.44±1.37 per cent, 4.49±0.18 x 10^6/cmm, 13.24±0.75 x 10^3/cmm, 2.66±0.11 lakhs/cmm, 73.82±1.06 per cent, 19.71±0.81 per cent, 4.06±0.20 per cent and 3.27±0.17 per cent respectively. In dogs Group III with MVD the values were 12.30±0.40g/dl, 41.80±1.35 per cent, 5.07±0.21 x 10^6/cmm, 12.27±0.71 x 10^3/cmm, 2.65±0.14 lakhs/cmm, 73.76±0.92 per cent, 19.21±0.85 per cent, 4.22±0.23 per cent and 3.08±0.21 per cent respectively.

In dogs Group IV with Pericardial Effusion the values were 12.25±1.42 g/dl, 43.65±5.96 per cent, 4.59±0.70 x 10^6/cmm, 12.67 ±0.47 x 10^3/cmm, 2.63±0.45 lakhs/cmm, 71.50±2.09 per cent, 20.67±2.42 per cent, 4.17±0.70 per cent and 3.67±0.67 per cent respectively.

In dogs Group V with HCM the values were 12.20±2.78 g/dl, 37.60±8.84 per cent, 4.41±0.32 x 10^6/cmm, 12.00±2.82 x 10^3/cmm, 2.41 ±0.29 lakhs/cmm, 72.33±3.84 per cent, 19.67±2.19 per cent, 3.50±0.50 per cent and 3.33±1.33 per cent respectively.

No statistically significant changes in the haematological values could be observed in control and AHD disease group.
<table>
<thead>
<tr>
<th>Haemogram</th>
<th>Group - I Control (n=20)</th>
<th>Group – II Dilated Cardiomyopathy (n=58)</th>
<th>Group – III Mitral Valve Disease (n=39)</th>
<th>Group – IV Pericardial Effusion (n=6)</th>
<th>Group – V Hypertrophic Cardiomyopathy (n=3)</th>
<th>F Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>12.84±1.46</td>
<td>12.41±0.37</td>
<td>12.30±0.40</td>
<td>12.25±1.42</td>
<td>12.20±2.78</td>
<td>1.020NS</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>40.92±4.44</td>
<td>40.44±1.37</td>
<td>41.80±1.35</td>
<td>43.65±5.96</td>
<td>37.60±8.84</td>
<td>2.285NS</td>
</tr>
<tr>
<td>RBC (m/cmm)</td>
<td>5.49±0.42</td>
<td>4.49±0.18</td>
<td>5.07±0.21</td>
<td>4.59±0.70</td>
<td>4.41±0.32</td>
<td>1.990NS</td>
</tr>
<tr>
<td>WBC (10³/cmm)</td>
<td>12.76±1.60</td>
<td>13.24±0.75</td>
<td>12.27±0.71</td>
<td>12.67±0.47</td>
<td>12.00±2.82</td>
<td>0.923NS</td>
</tr>
<tr>
<td>Platelets (lakhs/cmm)</td>
<td>2.47±0.61</td>
<td>2.66±0.11</td>
<td>2.65±0.14</td>
<td>2.63±0.45</td>
<td>2.41±0.29</td>
<td>0.245NS</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>73.00±2.58</td>
<td>73.82±1.06</td>
<td>73.76±0.92</td>
<td>71.50±2.09</td>
<td>72.33±3.84</td>
<td>1.896NS</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>18.00±2.37</td>
<td>19.71±0.81</td>
<td>19.21±0.85</td>
<td>20.67±2.42</td>
<td>19.67±2.19</td>
<td>1.562NS</td>
</tr>
<tr>
<td>Monocytes</td>
<td>3.25±0.70</td>
<td>4.06±0.20</td>
<td>4.22±0.23</td>
<td>4.17±0.70</td>
<td>3.33±1.33</td>
<td>0.858NS</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>3.08±0.36</td>
<td>3.27±0.17</td>
<td>3.08±0.21</td>
<td>3.67±0.67</td>
<td>3.50±0.50</td>
<td>0.742NS</td>
</tr>
</tbody>
</table>

Same superscript in row do not differ significantly
NS Not significant (P>0.05)
* Significant (P < 0.05)
** Highly Significant (P<0.01)
4.5 BIOCHEMISTRY

The mean ± SE values of studied biochemical parameters are presented in Table-IV. The mean ± SE biochemical parameter of healthy dogs in the present study had mean values of Blood Urea Nitrogen was 20.03±1.65 mg/dl, Creatinine was 1.46±0.15 mg/dl, ALT was 49.86±15.92 IU/dl, ALP were 171.89±29.99 IU/dl, Total Protein was 6.14±0.24 g/dl, Albumin was 2.49±0.07 g/dl, Calcium was 9.92±0.35 mmol/dl, Phosphorous was 5.34±0.59 mmol/dl, Sodium was 148.78±4.19 mmol/dl and Potassium was 4.70±0.09 mmol/dl respectively.

Group II dogs with DCM had mean ± SE serum Blood Urea Nitrogen, Creatinine, ALT, ALP, Total Protein, Albumin, Calcium, Phosphorous, Sodium and Potassium were 19.86±1.07 mg/dl, 1.08±0.06 mg/dl, 60.70±3.75 IU/dl, 121.04±13.35 IU/dl, 6.42±0.12 g/dl, 2.66±0.07 g/dl, 10.25±0.15 mmol/dl, 4.24±0.21 mmol/dl, 157.93±1.52 mmol/dl and 4.81±0.04 mmol/dl respectively.

Group III dogs with MVD had mean±SE serum Blood Urea Nitrogen, Creatinine, ALT, ALP, Total Protein, Albumin, Calcium, Phosphorous, Sodium and Potassium were 20.72±2.99 mg/dl, 1.36±0.32 mg/dl, 59.03±4.78 IU/dl, 136.50±30.55 IU/dl, 6.55±0.14 g/dl, 2.74±0.07 g/dl, 9.71±0.22 mmol/dl, 4.25±0.24 mmol/dl, 162.13±1.47 mmol/dl and 4.78±0.04 mmol/dl respectively.

In Group IV with Pericardial Effusion the mean±SE serum Blood Urea Nitrogen, Creatinine, ALT, ALP, Total Protein, Albumin, Calcium, Phosphorous, Sodium and Potassium were 18.34±2.92 mg/dl, 1.09±0.17 mg/dl, 105.00±62.38 IU/dl, 160.33±40.50 IU/dl, 6.56±0.31 g/dl, 2.36±0.24 g/dl, 8.98±0.89 mmol/dl, 3.37±0.31 mmol/dl, 157.00±5.05 mmol/dl and 4.76±0.12 mmol/dl respectively.

In Group V with HCM the mean±SE serum Blood Urea Nitrogen, Creatinine, ALT, ALP, Total Protein, Albumin, Calcium, Phosphorous, Sodium and Potassium were 20.69±6.68 mg/dl, 1.03±0.25 mg/dl, 56.50±35.50 IU/dl, 347.00±231.13 IU/dl, 6.25±0.60 g/dl, 2.36±0.65 g/dl, 10.56±1.76 mmol/dl, 4.46±1.86 mmol/dl, 145.67±0.67 mmol/dl and 5.03±0.11 mmol/dl respectively.

Non-significant alterations were observed in the values of serum biochemistry in all groups when compare with control group except sodium. Sodium has a highly significant increase in DCM, MVD and Pericardial Effusion groups.
Table-IV
Mean ± SE Serum Biochemical Values in Control and Acquired Heart Disease Cases

<table>
<thead>
<tr>
<th>Serum Biochemistry</th>
<th>Group - I Control (n=20)</th>
<th>Group - II Dilated Cardiomyopathy (n=58)</th>
<th>Group – III Mitral Valve Disease (n=39)</th>
<th>Group – IV Pericardial Effusion (n=6)</th>
<th>Group – V Hypertrophic Cardiomyopathy (n=3)</th>
<th>F Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN (mg/dl)</td>
<td>20.03±1.65</td>
<td>19.86±1.07</td>
<td>20.72±2.99</td>
<td>18.34±2.92</td>
<td>20.69±6.68</td>
<td>0.060NS</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.46±0.15</td>
<td>1.08±0.06</td>
<td>1.36±0.32</td>
<td>1.09±0.17</td>
<td>1.03±0.25</td>
<td>0.438NS</td>
</tr>
<tr>
<td>ALT (IU/dl)</td>
<td>49.86±15.92</td>
<td>60.70±3.75</td>
<td>59.03±4.78</td>
<td>105.00±62.38</td>
<td>56.50±35.50</td>
<td>1.589NS</td>
</tr>
<tr>
<td>ALP (IU/dl)</td>
<td>171.89±29.99</td>
<td>121.04±13.35</td>
<td>136.50±30.55</td>
<td>160.33±40.50</td>
<td>347.00±231.13</td>
<td>1.930NS</td>
</tr>
<tr>
<td>TP (g/dl)</td>
<td>6.14±0.24</td>
<td>6.42±0.12</td>
<td>6.55±0.14</td>
<td>6.56±0.31</td>
<td>6.25±0.60</td>
<td>0.505NS</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.49±0.07</td>
<td>2.66±0.07</td>
<td>2.74±0.07</td>
<td>2.36±0.24</td>
<td>2.36±0.65</td>
<td>1.407NS</td>
</tr>
<tr>
<td>Calcium (mmol/dl)</td>
<td>9.92±0.35</td>
<td>10.25±0.15</td>
<td>9.71±0.22</td>
<td>8.98±0.89</td>
<td>10.56±1.76</td>
<td>1.970NS</td>
</tr>
<tr>
<td>Phosphorous (mmol/dl)</td>
<td>5.34±0.59</td>
<td>4.24±0.21</td>
<td>4.25±0.24</td>
<td>3.37±0.31</td>
<td>4.46±1.86</td>
<td>1.478NS</td>
</tr>
<tr>
<td>Sodium (mmol/dl)</td>
<td>148.78±4.19a</td>
<td>157.93±1.52b</td>
<td>162.13±1.47b</td>
<td>157.00±5.05b</td>
<td>145.67±0.67a</td>
<td>4.190**</td>
</tr>
<tr>
<td>Potassium (mmol/dl)</td>
<td>4.70±0.09</td>
<td>4.81±0.04</td>
<td>4.78±0.04</td>
<td>4.76±0.12</td>
<td>5.03±0.11</td>
<td>0.815NS</td>
</tr>
</tbody>
</table>

Same superscript in row do not differ significantly
NS Not significant (P>0.05)
* Significant (P < 0.05)
**Highly Significant (P<0.01)
4.6 RADIOGRAPHY

The Radiographic findings are presented in Table-V. The mean ± SE values of Vertebral Heart Score (VHS) for control group was 9.99±0.20. The VHS was a highly significantly increased in Group II (10.81±0.08) and Group IV (11.13±0.15), when compared to that of control group. Radiographic (Plate-IVa, b, c) evidence of cardiomegaly was observed in 93.10 per cent of Group II, 15.38 per cent of Group III and 100.00 per cent of Group IV. Radiographic evidence of pulmonary oedema was evident in 72.76 per cent of Group II, 71.79 per cent of Group III, 16.67 per cent of Group IV and 33.33 per cent of Group V.

Pleural effusion was evident in 20.69 per cent of Group II, 15.38 per cent of Group III and 16.67 per cent of Group IV. Radiographic evidence of left atrial enlargement was evident in 5.17 per cent of Group II and 82.05 per cent of Group III.

4.7 DOPPLER BLOOD PRESSURE

The mean ± SE values of systolic blood pressure are presented in Table-VI. No significant differences were observed in the mean ± SE values of systolic blood pressure in different groups of AHDs in dogs, when compared to that of the control group.

4.8 ELECTROCARDIOGRAPHY

The ECG findings are presented in Table-VII. All the control groups had normal sinus rhythm. Sinus Tachycardia was observed in 65.52%, 66.67%, 83.33%, and 100% in Group II, III, IV and V respectively.

Group II with DCM had characteristic ECG pattern of atrial fibrillation in 20.69 per cent cases and atrial flutter in 8.62 per cent of cases. Group III with MVD had normal sinus rhythm in 33.33 per cent of cases. Group IV had normal sinus rhythm in 16.67 per cent of cases (Plate-V). 10.34 per cent of dogs in Group II with DCM had electrocardiographic evidence of atrial enlargement; while it was observed in Group III with MVD in 51.28 per cent. Low voltage QRS complex observed in Group II, Group III, Group IV 8.62 per cent, 7.69 per cent and 83.33 per cent respectively. Ventricular enlargement was observed to be 17.24 per cent in Group II and 100.00 per cent in Group V. Ventricular Premature Contraction was observed
### Table V
Radiographic Findings in Dogs with Acquired Heart Disease

<table>
<thead>
<tr>
<th>Radiographic Findings</th>
<th>Group - I Control (n=20)</th>
<th>Group – II Dilated Cardiomyopathy (n=58)</th>
<th>Group – III Mitral Valve Disease (n=39)</th>
<th>Group – IV Pericardial Effusion (n=6)</th>
<th>Group – V Hypertrophic Cardiomyopathy (n=3)</th>
<th>F Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertebral Heart Score (VHS)</td>
<td>9.99±0.20a</td>
<td>10.81±0.08b</td>
<td>9.71±0.11a</td>
<td>11.13±0.15b</td>
<td>9.70±0.38a</td>
<td>21.286**</td>
</tr>
<tr>
<td>Cardiomegaly (%)</td>
<td>-</td>
<td>93.10</td>
<td>15.38</td>
<td>100.00</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Pulmonary Oedema (%)</td>
<td>-</td>
<td>72.76</td>
<td>71.79</td>
<td>16.67</td>
<td>33.33</td>
<td></td>
</tr>
<tr>
<td>Pleural effusion (%)</td>
<td>-</td>
<td>20.69</td>
<td>15.38</td>
<td>16.67</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Left Atrial enlargement (%)</td>
<td>-</td>
<td>5.17</td>
<td>82.05</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

### Table VI
Doppler Systolic Blood Pressure in Control and Acquired Heart Disease Cases

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group - I Control (n=20)</th>
<th>Group – II Dilated Cardiomyopathy (n=58)</th>
<th>Group – III Mitral Valve Disease (n=39)</th>
<th>Group – IV Pericardial Effusion (n=6)</th>
<th>Group – V Hypertrophic Cardiomyopathy (n=3)</th>
<th>F Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP (mmHg)</td>
<td>115.56±3.38a</td>
<td>120.18±1.10a</td>
<td>120.00±1.41a</td>
<td>120.00±3.65a</td>
<td>120.00±5.77a</td>
<td>0.579NS</td>
</tr>
</tbody>
</table>

Same superscript in row do not differ significantly
NS Not significant (P>0.05)
*aSignificant (P < 0.05)
**Highly Significant (P<0.01)
<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>ECG findings</th>
<th>Group - I Control (n=20)</th>
<th>Group – II Dilated Cardiomyopathy (n=58)</th>
<th>Group – III Mitral Valve Disease (n=39)</th>
<th>Group – IV Pericardial Effusion (n=6)</th>
<th>Group – V Hypertrophic Cardiomyopathy (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I. Rhythm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal Sinus Rhythm</td>
<td>100.00%</td>
<td>5.17%</td>
<td>33.33%</td>
<td>16.67%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sinus Tachycardia</td>
<td>-</td>
<td>65.52%</td>
<td>66.67%</td>
<td>83.33%</td>
<td>100.00%</td>
</tr>
<tr>
<td></td>
<td>Atrial fibrillation</td>
<td>-</td>
<td>20.69%</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Atrial flutter</td>
<td>-</td>
<td>8.62%</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III. Complex morphology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1. Atrial enlargement</td>
<td>-</td>
<td>10.34%</td>
<td>51.28%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2. Low Voltage QRS Complex</td>
<td>-</td>
<td>8.62%</td>
<td>7.69%</td>
<td>83.33%</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3. Ventricular enlargement</td>
<td>-</td>
<td>17.24%</td>
<td>-</td>
<td>-</td>
<td>100.00%</td>
</tr>
<tr>
<td></td>
<td>4. Ventricular premature contraction</td>
<td>-</td>
<td>17.24%</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5. Electrical alternans</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>83.33%</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6. ST changes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. ST coving</td>
<td>-</td>
<td>13.79%</td>
<td>2.56%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>b. ST elevation</td>
<td>-</td>
<td>5.17%</td>
<td>2.56%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>c. ST depression</td>
<td>-</td>
<td>10.34%</td>
<td>2.56%</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
in Group II with DCM 17.24 per cent of cases. Electrical alternans observed in 83.33 per cent cases of Group IV with Pericardial Effusion. The observed ST changes in dogs with AHD included ST coving, ST elevation and ST depression in varying degrees.

4.9 ECHOCARDIOGRAPHY

4.9.1 Two-dimensional echocardiography

The Mean±S.E values of two dimensional echocardiography values in control and AHD dogs are given in Table-VIIIa & b.

Mean±S.E values of LA, Ao, LA/Ao, Simpson EDV, Simpson ESV, Simpson SV and Simpson EF in control dogs were 2.15±0.18 cm, 2.10±0.14 cm, 1.03±0.07 cm, 41.00±3.86 cm, 18.67±1.67 cm, 22.17±2.61 cm and 54.30±2.29 per cent respectively.

Mean±S.E values of LA, Ao, LA/Ao, Simpson EDV, Simpson ESV, Simpson SV and Simpson EF in Group II with DCM dogs were 4.10±0.11 cm, 2.30±0.04 cm, 1.82±0.06b cm, 79.31±7.60 cm, 52.70±6.58 cm, 26.31±4.77 cm and 34.30±5.36 per cent respectively (Plate-VI).

Mean±S.E values of LA, Ao and LA/Ao in Group III with MVD dogs were 3.42±0.20 cm, 1.79±0.07 cm and 1.93±0.09 cm per cent respectively.

Mean±S.E values of LA, Ao and LA/Ao, in Group IV with Pericardial Effusion dogs were 3.80±0.5 cm, 1.85±0.05 cm and 2.03±0.21 cm per cent respectively.

Mean±S.E values of LA, Ao and LA/Ao, in Group V with HCM dogs were 2.05±0.05 cm, 1.75±0.15 cm and 1.21±0.13 cm per cent respectively.

A highly significant increase in the mean±S.E values of LA, LA/Ao, Simpson EDV, Simpson ESV were noticed in Group I with DCM. A highly significant decrease in the Simpson EF was noticed in DCM dogs. A highly significant increase in the mean±S.E values of LA and LA/Ao were noticed in Group III with MVD and Group IV with Pericardial Effusion were observed. In Group V with HCM a highly significant decrease in LA and increase in LA/Ao were observed.
### Table-VIII
#### a. Mean ± SE Values of 2D-Echocardiography in Control and Acquired Heart Disease Cases

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group - I Control (n=20)</th>
<th>Group - II Dilated Cardiomyopathy (n=58)</th>
<th>Group – III Mitral Valve Disease (n=39)</th>
<th>Group – IV Pericardial Effusion (n=6)</th>
<th>Group – V Hypertrophic Cardiomyopathy (n=3)</th>
<th>F Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA (cm)</td>
<td>2.15±0.18&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.10±0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.42±0.20&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.80±0.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.05±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.974&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ao (cm)</td>
<td>2.10±0.14</td>
<td>2.30±0.04</td>
<td>1.79±0.07</td>
<td>1.85±0.05</td>
<td>1.75±0.15</td>
<td>10.623&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>LA/Ao(0.83-1.13)</td>
<td>1.03±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.82±0.06&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.93±0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.03±0.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.21±0.13&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.150&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

#### b. Mean ± SE Values of 2D-Echocardiography in Control and Acquired Heart Disease Cases by Simpson’s method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group - I Control</th>
<th>Group – II Dilated Cardiomyopathy</th>
<th>F Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simpson EDV (ml)</td>
<td>41.00±3.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.31±7.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.010&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Simpson ESV (ml)</td>
<td>18.67±1.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.70±6.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.097&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Simpson SV (ml)</td>
<td>22.17±2.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.31±4.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.634&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Simpson EF</td>
<td>54.30±2.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.30±5.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.578&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Same superscript in row do not differ significantly
NS Not significant (P>0.05)
*Significant (P < 0.05)
**Highly Significant (P<0.01)
In this study, when comparing disease groups with control group a highly significant increase in LA and LA/Ao were observed in DCM, MVD and pericardial effusion group.

4.9.2 M-mode echocardiography

The Mean±S.E values of M-mode echocardiography in control and DCM dogs are given in Table-IX.

Mean±S.E values of RVIDd, RVIDs, IVSd, IVSs, LVIDd, LVIDs, LVPWd, LVPWs, FS and EPSS in control dogs were 0.98±0.18 cm, 1.09±0.20 cm, 0.80±0.07 cm, 1.15±0.07 cm, 4.09±0.23 cm, 2.64±0.24 cm, 0.83±0.06 cm, 1.39±0.07 cm, 36.06±2.64 per cent and 0.73±0.07 cm per cent respectively.

Mean±S.E values of RVIDd, RVIDs, IVSd, IVSs, LVIDd, LVIDs, LVPWd, LVPWs, FS and EPSS in Group II with DCM dogs were 1.16±0.08 cm, 1.40±0.09 cm, 0.78±0.03 cm, 0.96±0.04 cm, 5.72±0.11 cm, 4.79±0.09 cm, 0.80±0.03 cm, 1.12±0.04 cm, 16.30±0.76 per cent and 1.77±0.13 cm per cent respectively (Plate-VIb).

Mean±S.E values of RVIDd, RVIDs, IVSd, IVSs, LVIDd, LVIDs, LVPWd, LVPWs, FS and EPSS in Group III with MVD dogs were 0.80±0.09 cm, 0.88±0.08 cm, 0.74±0.03 cm, 1.13±0.05 cm, 3.79±0.19 cm, 2.26±0.15 cm, 0.79±0.03 cm, 1.32±0.05 cm, 42.47±2.04 per cent, 0.70±0.20 cm respectively.

Mean±S.E values of RVIDd, RVIDs, IVSd, IVSs, LVIDd, LVIDs, LVPWd, LVPWs, and FS in Group IV with pericardial effusion were 1.08±0.12 cm, 1.14±0.28 cm, 0.92±0.07 cm, 1.12±0.07 cm, 3.78±0.29 cm, 2.42±0.20 cm, 0.74±0.07 cm, 1.14±0.07 cm and 34.94±5.47 per cent respectively.

Mean±S.E values of RVIDd, RVIDs, IVSd, IVSs, LVIDd, LVIDs, LVPWd, LVPWs and FS in Group V with hypertrophic cardiomyopathy dogs were 0.57±0.09 cm, 0.53±0.03 cm, 0.83±0.07 cm, 1.37±0.12 cm, 2.07±0.84 cm, 1.10±0.56 cm, 1.30±0.32 cm, 1.67±0.18 cm and 51.37±5.21 per cent respectively.

The fractional thickening M-mode indices of IVSFT, LVPWFT in control, DCM, MVD, Pericardial effusion and HCM were 61.43±22.50, 35.05±2.94, 56.74±5.97, 33.00±10.08, 65.67±16.50; and 71.75±9.30, 46.94±4.25, 70.21±5.76, 60.20±17.80, 37.67±20.34 respectively.
In Group II with DCM a highly significant increase in the mean values of RVIDs, LVIDd, LVIDs and EPSS; and a significant increase in RVIDd were noticed, where as a highly significant decrease in IVSs, LVPWd, LVPWs and FS; and significant decrease in IVSFT and LVPWFT were noticed.

In Group III with MVD a highly significant increase in the mean values of FS (supernormal); and a highly significant decrease in RVIDs, IVSs, LVIDd, LVIDs, LVPWd, LVPWs, EPSS; and significant decrease in RVIDd, IVSFT and LVPWFT were noticed.

In Group IV with pericardial effusion a highly significant increase in the mean values of RVIDs; and a significant increase in RVIDd were noticed, where as a highly significant decrease in IVSs, LVIDd, LVIDs, LVPWd, LVPWs and FS; and significant decrease in IVSFT and LVPWFT were noticed.

In Group V with HCM a highly significant increase in IVSs, LVPWd, LVPWs and FS were observed. In addition a significant increase in IVSFT was noticed. A highly significant decrease in RVIDs, LVIDd, LVIDs; and significant decrease in RVIDd and LVPWFT noticed in this group.

In this study when comparing the disease groups with control group a highly significant increase in LVIDd, LVIDs and EPSS of DCM group, FS (supernormal) of MVD and HCM group, LVPWd of HCM group; and a highly significant decrease in FS in DCM group; and LVIDd & LVIDs of HCM group were the classical and important findings.

4.9.3 Colour Flow Doppler Echocardiography

In the control group normal flow pattern were observed in Left Atrium, Right Atrium, Aorta, Pulmonary Artery, Mitral Valve and Tricuspid Valve (Figure-VI).

In Group II with DCM group no change in the flow pattern were observed across Ao and PA in any of the dogs, where as normal flow pattern across MV and TV were observed only in 13.79 per cent of dogs. Mitral valve regurgitation were noticed in 86.21 per cent of dogs and Tricuspid valve regurgitation were noticed in 34.48 per cent of dogs. Both mitral and tricuspid valve regurgitation were noticed in 31.03 per cent of dogs (Plate-VIc).
Table-IX
Mean ± SE Values of M-Mode Echocardiography in Control and Acquired Heart Disease Cases

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group - I Control (n=20)</th>
<th>Group – II Dilated Cardiomyopathy (n=58)</th>
<th>Group – III Mitral Valve Disease (n=39)</th>
<th>Group – IV Pericardial Effusion (n=6)</th>
<th>Group – V Hypertrophic Cardiomyopathy (n=3)</th>
<th>F Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RVIDd (cm)</td>
<td>0.98±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.16±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.80±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.08±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.57±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.780&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>RVIDs (cm)</td>
<td>1.09±0.20&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.40±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.88±0.08&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.14±0.28&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.53±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.192&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>IVSd (cm)</td>
<td>0.80±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.78±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.74±0.03&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.92±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.83±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.061&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>IVSs (cm)</td>
<td>1.15±0.07&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.96±0.04&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.13±0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.12±0.07&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.37±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.199&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>IVSFT (%)</td>
<td>61.43±22.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.05±2.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.74±5.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.00±10.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.67±16.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.183&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>LVIDd (cm)</td>
<td>4.09±0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.72±0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.79±0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.78±0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.07±0.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.749&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>LVIDs (cm)</td>
<td>2.64±0.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.79±0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.26±0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.42±0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.10±0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.077&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>LVPIWd (cm)</td>
<td>0.83±0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.80±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.79±0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.74±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.30±0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.165&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>LPWVs (cm)</td>
<td>1.39±0.07&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.12±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.32±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.14±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.67±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.360&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>LVPWFT (%)</td>
<td>71.75±9.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.94±4.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.21±5.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.20±17.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.67±20.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.427&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>FS (%)</td>
<td>36.06±2.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.30±0.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.47±2.04&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>34.94±5.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51.37±5.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>53.125&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>EPSS (cm)</td>
<td>0.73±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.77±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.70±0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>5.510&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Same superscript in row do not differ significantly
NS Not significant (P>0.05)
*Significant (P < 0.05)
**Highly Significant (P<0.01)
Figure VI

Colour Flow Doppler Echocardiographic findings in AHDs

- Mitral valve regurgitation: 86.21%
- Tricuspid regurgitation: 16.67%
- Aortic regurgitation: 34.48%
- Pulmonary Artery regurgitation: 0%

- Dilated Cardiomyopathy: 100.00%
- Mitral Valve Disease:%
- Pericardial Effusion: 0%
In Group III with MVD dogs Mitral valve regurgitation were noticed in 100.00 per cent of dogs with normal flow pattern in Aorta and Pulmonary Artery. In Group IV with Pericardial Effusion the MR were present in 16.67 per cent

4.10 TISSUE DOPPLER IMAGING (TDI)

4.10.1 Pulsed wave Tissue Doppler Imaging (PW-TDI)

The Mean±S.E values of PW-TDI at mitral annulus in control and AHD dogs are given in Table-X.

Mean±S.E values of Sm, Em, Am, Em/Am, IVRT and IVCT of control animals were 0.16±0.02 m/sec, 0.16±0.01 m/sec, 0.08±0.00 m/sec, 2.05±0.20, 81.80±25.11 ms and 35.60±9.89 ms respectively.

Mean±S.E values of Sm, Em, Am, Em/Am, IVRT and IVCT of Group II with DCM animals were 0.10±0.00 m/sec, 0.12±0.01 m/sec, 0.08±0.01 m/sec, 1.98±0.14, 93.53±16.37 ms and 81.59±10.22 ms respectively (Plate-VII).

Mean±S.E values of Sm, Em, Am, Em/Am, IVRT and IVCT of Group III with MVD animals were 0.12±0.01 m/sec, 0.14±0.01 m/sec, 0.08±0.01 m/sec, 1.81±0.14, 103.08±16.08 ms and 111.62±22.95 ms respectively.

Mean±S.E values of Sm, Em, Am, Em/Am, IVRT and IVCT of Group IV with pericardial effusion animals were 0.13±0.01 m/sec, 0.09±0.01 m/sec, 0.12±0.01 m/sec, 0.79±0.20, 62.00±28.58 ms and 57.00±23.12 ms respectively.

Mean±S.E values of Sm, Em, Am, Em/Am, IVRT and IVCT of Group V with hypertrophic cardiomyopathy animals were 0.11±0.03 m/sec, 0.09±0.04 m/sec, 0.12±0.02 m/sec, 0.69±0.16, 152.00±24.00 ms and 66.50±13.50 ms respectively.

A highly significant decrease in Sm and Em/Am ratio all groups; and a significant decrease in Em in all groups were observed; and a significant increase in Am in pericardial effusion and HCM were observed. Very typical Em/Am ratio reversal were observed in pericardial effusion and HCM group.

In this study in HCM group even though there were no significant changes observed in IVRT it were relatively higher than the other groups.
### Table-X

Mean ± SE Values of Pulsed Wave -Tissue Doppler Imaging in Control and Acquired Heart Disease Cases

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group - I Control (n=20)</th>
<th>Group – II Dilated Cardiomyopathy (n=58)</th>
<th>Group – III Mitral Valve Disease (n=39)</th>
<th>Group – IV Pericardial Effusion (n=6)</th>
<th>Group – V Hypertrophic Cardiomyopathy (n=3)</th>
<th>F Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sm (m/sec)</td>
<td>0.16±0.02</td>
<td>0.10±0.00</td>
<td>0.12±0.01</td>
<td>0.13±0.01</td>
<td>0.11±0.03</td>
<td>5.591**</td>
</tr>
<tr>
<td>Em (m/sec)</td>
<td>0.16±0.01</td>
<td>0.12±0.01</td>
<td>0.14±0.01</td>
<td><strong>0.09±0.01</strong></td>
<td>0.09±0.04</td>
<td>2.935*</td>
</tr>
<tr>
<td>Am (m/sec)</td>
<td>0.08±0.00</td>
<td>0.08±0.01</td>
<td>0.08±0.01</td>
<td>0.12±0.01</td>
<td>0.12±0.02</td>
<td>3.690*</td>
</tr>
<tr>
<td>Em/Am</td>
<td>2.05±0.20</td>
<td>1.98±0.14</td>
<td>1.81±0.14</td>
<td><strong>0.79±0.20</strong></td>
<td>0.69±0.16</td>
<td>5.790**</td>
</tr>
<tr>
<td>IVRT (ms)</td>
<td>81.80±25.11</td>
<td>93.53±16.37</td>
<td>103.08±16.08</td>
<td>62.00±28.58</td>
<td>152.00±24.00</td>
<td>0.763NS</td>
</tr>
<tr>
<td>IVCT (ms)</td>
<td>35.60±9.89</td>
<td>81.59±10.22</td>
<td>111.62±22.95</td>
<td>57.00±23.12</td>
<td>66.50±13.50</td>
<td>1.845NS</td>
</tr>
<tr>
<td>Global TDI Velocity</td>
<td>0.33±0.08</td>
<td><strong>0.17±0.02</strong></td>
<td><strong>0.20±0.02</strong></td>
<td><strong>0.10±0.03</strong></td>
<td><strong>0.08±0.04</strong></td>
<td>4.984**</td>
</tr>
</tbody>
</table>

Same superscript in row do not differ significantly
NS Not significant (P>0.05)
*Significant (P<0.05)
**Highly Significant (P<0.01)
In the present study a highly significant increase in Am velocity; and a highly significant decrease in Em and Em/Am ratio were observed in pericardial effusion and HCM groups.

4.10.2 Global TDI Velocity

The Mean±S.E values of Global TDI velocity in control and AHD dogs are given in Table-X.

The Mean±S.E values of Global TDI velocity in DCM, MVD, pericardial effusion and HCM were 0.33±0.08, 0.17±0.02, 0.20±0.02, 0.10±0.03 and 0.08±0.04. A highly significant decrease were observed in all groups when compared to control group.

4.10.3 2D-Colour TDI

As with conventional Doppler system, tissue velocities towards the transducer were colored in red, and away from the transducer were colored in blue. The 2 dimensional TDI images during systole and diastole at mitral annulus, inter ventricular septum and left ventricular posterior wall are presented in the Plates-VII.

4.10.4 Colour M-mode

Due to a high sampling rate, M-mode might detect subtle changes in myocardial motion velocities. By comparison with 2D TDI imaging, M-mode TDI affords a much higher temporal resolution and also greater signal-to-noise ratio. M-mode TDI tracings show on the same image both systolic and diastolic velocities within the entire wall thickness. As with the 2D color TDI mode, myocardial velocities toward the transducer were encoded in red, and those away from the transducer in blue. Systole started with the short isovolumic contraction phase, followed by the ejection phase with higher velocities. During the diastole, 4 phases identified: diastole started with a short isovolumic relaxation phase followed by the early filling phase with much higher velocities. The third phase with lower velocities were diastasis, followed by the late filling phase with higher velocities (therefore brighter colors) during atrial contraction.
<table>
<thead>
<tr>
<th>Marker</th>
<th>Control (n=6)</th>
<th>Dilated Cardiomyopathy (n=12)</th>
<th>Mitral Valve Disease with Systolic failure (n=7)</th>
<th>Mitral Valve Disease without Systolic failure (n=5)</th>
<th>Occult Cardiac disease (n=3)</th>
<th>Others⁷ (n=7)</th>
<th>F Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT proBNP Assay (pmol/L)</td>
<td>650.00±30.55a</td>
<td>3041.67±65.67d</td>
<td>2000.00±127.24c</td>
<td>1080.00±177.20b</td>
<td>1233.33±240.37b</td>
<td>604.28±37.98a</td>
<td>117.12**</td>
</tr>
</tbody>
</table>

Same superscript in row do not differ significantly
NS Not significant (P>0.05)
*Significant (P < 0.05)
**Highly Significant (P<0.01)

⁷Others – These animals shown the clinical signs of suggestive of cardiac disease but not shown any cardiac dysfunction in echocardiography
4.11 NT-proBNP MARKER ASSAY

The Mean±S.E values of NT proBNP assay in control and AHD dogs are given in Table-XI.

The Mean±S.E values of NT proBNP assay in control, DCM, MVD with systolic failure, MVD without systolic failure, Occult cardiac disease and non-cardiac cases were 650.00±30.55 pmol/L, 3041.67±65.67 pmol/L, 2000.00±127.24 pmol/L, 1080.00±177.20 pmol/L, 1233.33±240.37 pmol/L and 604.28±37.98 pmol/L respectively. A highly significant increase were observed in Dilated Cardiomyopathy, MVD with systolic failure, MVD without systolic failure and Occult cardiac disease (Plate-VIII).

4.12 POST-MORTEM EXAMINATION

The post-mortem examination were performed in 4 dogs with DCM that were died during the study period. Their findings were summarized as follows.

4.12.1 Gross pathology

In DCM dogs heart were enlarged, globular, and flabby in appearance with rounded apex due to generalized ventricular dilatation. On transverse biventricular slice of heart the marked dilatation of lumen, attenuation of papillary muscles and thinning of free walls of ventricles were observed (Plate-IXa)

4.12.2 Histopathology

The histopathology of left ventricular myocardium of DCM showed attenuated wavy fibre type of myofibre with thinner, well separated and wavy appearance were observed in four dogs. Focal myocarditis and necrosis were present with infiltration of collagen material in the myocardial fibres (Plate-IXb). Focal myocarditis and necrosis were observed by collagen deposition in the cardiac myofibre with help of Picro Sirius Red staining method.
Acquired Heart Diseases (AHD)

AHDs are the common cardiac diseases in dogs in small, medium and large breed dogs. The common AHDs in dogs were dilated cardiomyopathy, mitral valve disease, and pericardial effusion. In this, DCM is very common in medium and large breed dogs and MVD is common in small and toy breeds. In India, more reports available in systolic failure due to myocardial and valvular diseases and very few were available on diastolic failures. In this study apart from systolic failure equal emphasis were given on diastolic failure to identify the significance in diagnosis and treatment of AHDs. In addition the efficacy of cardiac bio-marker NT-proBNP were evaluated in diagnosing and categorising the cardiac diseases. The cases presented to MVC teaching hospital were classified into DCM, MVD, pericardial effusion and HCM based on the diagnostic tests carried out.

5.1 PREVALENCE

The prevalence of AHDs is presented in Fig-I and II. In the present study the prevalence of AHDs were found to be 0.37 per cent. This is in agreement with Fisher et al. (2001), who reported CHF as one among the most frequent encountered cardiac disease, with estimated prevalence of one per cent.

5.1.1 Breed

The breed wise incidences of AHDs are presented in Fig-III-a, III-b and III-c. The higher prevalence of DCM in Labrador retrievers (28 out of 58) in this study may be due to the preference of this breed by dog owners of Chennai or due to mode of inheritance from individual in breeding. McEwan (2000) opined that in the most of the familial DCM, autosomal dominant mode of inheritance were suspected, whereas Ortiz-Lopez et al. (1996) considered autosomal dominant, autosomal recessive, X-linked and mitochondrial modes of inheritance in the DCM affected dogs. Therefore the ancestral population would have been heterozygotes or homozygotes for DCM related inheritance. Very high incidence
of around 24 per cent of DCM were observed by Vollmar (2000) in Irish wolfhounds. Tidholm et al. (1997) found that DCM was commonly reported in large and giant breed dogs such as Doberman Pinschers, Boxers and Great Danes. In Chennai most of the dog owners prefer Labrador as their pet which could be the reason for the high incidence. Hence more study is required to prove the genetic inheritance of DCM in Labrador.

Das and Tashjian, (1965), Detweiler and Patterson, (1965) and (Buchanan, 1977) reported the prevalence of valvular incompetence leading to heart failure were higher in certain small and medium sized breeds including Poodle, Schnauzer, Chihuahua, Doberman Pinscher, Fox Terrier, Boston Terrier and Cocker Spaniel. Abbott (1998) reported CHF resulting from MVD were observed almost exclusively in geriatric small breed dogs. In our study the MVD were found very commonly in Spitz (17/39) compared to other breeds. This finding is in accordance with the previous findings.

5.1.2 Age

The age wise prevalence is presented in Fig-IV. The average age in years of the affected dog were 6.48±0.40 in DCM, 9.00±0.59 in MVD, 8.17±0.79 in pericardial effusion and 6.17±2.35 in HCM. This concurs with the findings of Tidholm et al. (1997) where they observed DCM at a mean age of 6.6 years. Similar observations were also made by various authors Tidholm et al. (1997); Calvert et al. (1997); Meurs, (1998); Meurs et al. (2001); Petric et al. (2002) and Meurs et al. (2007). Detweiler et al. (1968); Sisson and Thomas, (1995); Tidholm and Jonsson, (1996); and Häggström et al. (2000) noted the increased prevalence of the MVD with older age group. Highest prevalence were noticed in age group of 8-10 years in the present study followed by 6-8 years group, and this concurs with the observation of above authors.

5.1.3 Sex

The sex wise prevalence is presented in Fig-V. The incidence of AHDs in male dogs were higher compared to females in the present study. DCM were more common in males than females as per the findings of Tidholm and Jonsson (1996),
Tidholm et al. (1997), Calvert et al. (1997), O’Grady and Horne (1998), Vollmar (1999), Petric et al. (2002), Martin et al. (2010). Detweiler and Patterson (1965) and Thrusfield et al. (1985) reported MVD is more common in males. The findings of the present study showed male predominance in all acquired heart diseases. However in our study, the predominance of male dogs may be due to the over representation of males in the population which reflected the male predominance which were also observed by earlier workers at Madras Veterinary College (Arun, 2012; Sivaprakasam, 2012).

5.2 MEDICAL HISTORY

The nature of presenting complaints is presented in Table-I.

In the present study the major history and clinical signs in DCM were abdominal distension, exercise intolerance, weight loss, persistent cough, weakness, dyspnoea and syncope. The above findings are in agreement with Fisher, (1972); Tidholm et al., (1997); Bulmer, (2006); Erling and MazzaFerro, (2008); and Fox, (1988).

In MVD persistent cough might be due to compression of left main stem brochi the major finding in our study, which is similar to the findings of Bonagura and Frank (1983), Kittleson and Kienle (1998) and Kvart and Häggström (2000).

In HCM group, syncope alone were the predominant complaint. This concurs with Thomas, (1987). In pericardial effusion group, signs were similar to DCM group. History reported in the study may be attributed to systolic and/or diastolic failure, dilated left atrium might be due to regurgitation; pulmonary oedema might be due to poor cardiac performance and circulatory collapse might be due to reduced cardiac output. Major study findings are in agreement with many authors (Meurs et al., 2001; Martin et al., 2009; and Martin et al., 2010; Darke, 1985; Tidholm and Jonsson, 2005; and Martin et al., 2009).

5.3 CLINICAL PRESENTATION

The clinical findings in different groups of AHDs are presented in Table-II.
The predominant physical examination findings in the present study were tachycardia, murmur, pulmonary oedema and ascites followed by other findings like pedal oedema and pulse deficit might be due to atrial fibrillation in DCM group. Murmur might be due to regurgitation secondary to dilatation; pulmonary edema might be due to poor cardiac performance; and ascites might be due to increased sodium and water retention in cardiac cases. These findings concurs with several authors (Häggström et al. 1995; Swenson et al., 1996; Tidholm and Jonsson, 1996; Tidholm et al. 1997; Jordon, 2003; Ristic, 2004; Martin et al., 2009; and Martin et al., 2010). Systolic murmur and low pitched pro diastolic (S3) gallop sound were auscultated as an evidence of severe ventricular diastolic impairment as reported by Sisson and Thomas, (1995).

In MVD group systolic murmur in different grades, honking cough, tachycardia, pulmonary oedema were the major clinical findings. Regurgitation might be the reason for murmurs; cough might be due to the compression of left main stem bronchi; and pulmonary oedema might be due to overworked left ventricle weren't able to pump out enough of the blood it received from lungs. These findings are similar to findings of Bonagura and Frank (1983); Häggström et al. (1995); and Häggström, (1996).

In pericardial effusion group the major findings were tachycardia, pulsus paradoxus, ascites and coughing. In HCM group tachycardia and pulse deficit due to very low cardiac output were major signs. In pericardial effusion and HCM the signs are mainly because of impairment in filling of ventricle and very low cardiac output. Majority of the signs were also noticed in various studies by Moise et al., (1986); Häggström et al. (1995); Swenson et al. (1996); French et al. (1998); Kittleson and Kienle, (1998); and Kovacic and Muller, (2003) and Shaw and Rush, (2007).

5.4 HAEMATOLOGY AND SERUM BIOCHEMISTRY

The Erythrogram and leucogram values are presented in Table-III.

In the current study haematological examination showed no significant changes in dogs with AHDs. It is similar to the findings of Tidholm and Jonsson
(1996) and Martin et al. (2009). These authors observed no haematological abnormalities in AHD dogs.

The mean ± SE values of studied biochemical parameters are presented in Table-IV. In the current study serum biochemical examination showed no significant changes in dogs with AHDs except sodium. A highly significant increase in Sodium values were observed in the present study in all groups except HCM. Andreoli, (1999) reported renal retention of sodium in heart failure were the major reason for water retention. This concurs with the above findings.

5.5 RADIOGRAPHY

The Radiographic findings are presented in Table-V. Radiographic findings were considered important as they provided information regarding the presence and severity of any underlying cardiac disease or related co-morbidities (Buchanan and Bucheler, 1995). Dogs with AHDs were found to have radiographic evidence of heart enlargement and particularly left atrial enlargement in MVD, pulmonary signs of congestive heart failure, which included increased pulmonary venous prominence, vascular and interstitial changes. In the current study, there were radiographic evidence of cardiomegaly (93.10 per cent) and pulmonary oedema (82.76 per cent) in dogs with DCM. These findings are in agreement with (Tidholm and Jonsson, 1996; Meurs et al., 2001 and Martin et al., 2009).

Radiographic evidence of left atrial enlargement were observed in majority of the dogs with MVD in the current study which concurs with various authors (Thomas, 1984; and Menaut et al., 2005).

The dogs with DCM had a highly significant elevated Vertebral Heart Score and radiographic signs that confirmed the presence of structural heart disease. This might be due to dilatation of all chambers in DCM. Similarly in pericardial effusion a highly significant increase in VHS were observed. This finding were mainly because of the dilation of pericardium with fluid. In radiographic findings of the present study the heart appeared like foot-ball with rounded margins. These findings are similar to the observations of Buchanan and Bucheler, (1995); Lister
and Buchanan, (2000); and Lamb et al., (2000). In hypertrophic cardiomyopathy there were no radiographic changes. This concurs with Fox (2003).

5.6 ELECTROCARDIOGRAPHY

The ECG findings are presented in Table-VII.

Major rhythm disturbances observed were sinus tachycardia (66.67 per cent), atrial fibrillation (20.69 per cent) and atrial flutter (8.62 per cent) of DCM group. The findings in the present study are in agreement with several authors (Calvert and Brown, 1986; Sisson and Thomas, 1995; Tidholm and Jonsson, 1996; Kittleson, 1998; Sisson et al., 1999; Calvert and Wall, 2001). They all observed atrial fibrillation as the common arrhythmias in dilated cardiomyopathy affected dogs across the breeds. Tidholm (2005) reported that atrial fibrillation in advanced DCM indicates poor prognosis. Saunders et al. (2009) reported that prognosis were guarded in the presence of dilated cardiomyopathy with atrial flutter in dogs.

Complex morphology findings were commonly observed in DCM, MVD, pericardial effusion and HCM groups. Ventricular enlargement (17.24 per cent), Ventricular premature contraction (17.24 per cent), ST coving (13.79 per cent), ST depression (10.34 per cent) and atrial enlargement (10.34 per cent) were the findings with DCM group. These findings are in agreement with Sisson and Thomas (1995); Brownlie (1991); Brownlie and Cobb (1999) and Vollmar (2000).

In MVD group the major finding were the atrial enlargement in 51.28 per cent cases. Low voltage QRS complex and electrical alternans were observed in 83.33 per cent cases in pericardial effusion. Reduction in QRS voltage were due to the volume of fluid surrounding the heart. The similar findings were reported by Tilley (1992); Häggström et al., (1992); and Darke et al. (1996).

Electrical alternans occurs because of enough effusion were present to enable beat-to-beat swinging of the heart within the pericardial space. Clarke (2002) and Bonagura (1981) reported the electrical alternans in dogs with
pericardial effusion. Ventricular enlargement (100.00 per cent) were the finding in HCM group and it were due LV hypertrophy.

5.7 SYSTOLIC BLOOD PRESSURE MEASUREMENT BY DOPPLER METHOD

The mean ± SE values of systolic blood pressure are presented in Table VI.

No significant difference in the mean systolic blood pressure were observed in AHD dogs compared to normal dogs. This finding is in agreement with Weiser et al. (1992) who reported normal systolic blood pressure.

Increased blood pressure increases the peripheral vascular resistance causing increase in the after load. Therefore increased blood pressure can also be a reason for reduced fractional shortening with normal left ventricular end diastolic dimension in healthy dogs. In this study the DCM group has reduced FS, which were mainly due to increased left ventricular end diastolic dimension, not due to blood pressure as reported by Henik et al. (2004). Therefore blood pressure measurement is warranted in all the dogs which show reduced fractional shortening to rule out the primary cause.

Increased blood pressure results in secondary HCM in dogs and in this study the blood pressure were normal. Hence the HCM reported in this study were not secondary HCM. This finding concurs with Thomas et al., 1984; and Littmen et al., 1988; Brown and Henik, 1998; De-Morais and Schwartz (2005).

5.8 ECHOCARDIOGRAPHY

5.8.1 Two dimensional echocardiography

The Mean±S.E values of two dimensional echocardiography values in control and AHD dogs are given in Table-VIII a & b.

In this study when comparing disease group with control a highly significant increase in LA and LA/Ao were observed in DCM and MVD; and significant increase in LA/Ao were observed in pericardial effusion.
A highly significant increase in the mean±S.E values of LA, LA/Ao, Simpson EDV, Simpson ESV were noticed in Group I with DCM. A highly significant decrease in the Simpson EF were noticed in DCM dogs. These findings are in partial agreement with Borgarelli et al. (2006) in a study of 63 dogs with DCM reported EF as 27.7 per cent. McEwan et al 2003 suggested that less than 40 per cent of EF determined by modified Simpson’s rule were abnormally low. In the present study, DCM dogs showed an ejection fraction of 34.30±5.36 per cent when compared to control dogs which is in agreement with the above study.

The Simpson’s method of disc (SMOD) evolves tracing of LV internal chambers and computerised calculations treating the ventricles as a stack of discs. In this method volume of each disc were calculated and summated for the total LV volume. This method shows best correlation with actual left ventricular volume in the diseased heart and appears relatively unaffected by changes in the ventricular geometry (Boon, 2011). The present study involved SMOD for calculating EF in Group II DCM dogs as described by above author were found to be more accurate.

A highly significant increase in the mean±S.E values of LA and LA/Ao were noticed in Group III with MVD and Group IV with Pericardial Effusion. Left atrial size was very similar to aortic root size in normal healthy dogs. A highly significant increase in LA/Ao ratio indicated continuous volume overload in left atrium in every systole. These findings concurs with findings of Chetboul and Tissier (2012).

A highly significant increase in the mean±S.E values of LA and LA/Ao were noticed in Group IV with Pericardial Effusion compared to control. The observed echocardiographic features of pericardial effusion were presence of an echo free or hypoechoic space between the epicardium and pericardial sac, swinging motion of the heart when the amount of fluid were significant and diastolic collapse of the right atrium or ventricle. The boundaries of pericardial effusion were smooth and conform to the shape of the heart. Pericardial effusion were typically not seen at the heart base below the atria while pleural effusion
were present. The size of the effusion does not determine whether tamponade was present. Diastolic collapse of the right atrium and/or ventricle confirms the presence of tamponade. In this study real time 2D imaging showed superior to M-mode in identifying effusion and tamponade. In case of pericardial effusion measured size were not a real indicator owing to tamponade. This finding concurs with Hoit (2007).

In Group V with HCM a highly significant decrease in LA and increase in LA/Ao were observed when comparing with other disease groups. When compared with control group these values were not significant. This slight change might be due to decrease in LA chamber size and size of the Aorta as reported by Liu et al. (1979) and Kovacic and Muller, (2003).

5.8.2 M-mode echocardiography

The Mean±S.E values of M-mode echocardiography in control and DCM dogs are given in Table-IX.

In Group II with DCM a highly significant increase in the mean values of RVIDs, LVIDd, LVIDs and EPSS; and a significant increase in RVIDd were noticed, where as a highly significant decrease in IVSs, LVPWd, LVPWs and FS; and a significant decrease in IVSFT and LVPWFT were noticed.

In the present study increased left ventricular dimensions in end diastole, end systole, thinning of interventricular septum and thinning of left ventricular posterior wall in diastole with reduced thickening of interventricular septum and left ventricular posterior wall during systole were observed. These findings concur with Vollmar (1999) who in a study of DCM in Irish wolfhounds reported similar findings. The presence or absence of left ventricular volume overload was determined from diastolic dimensions. This measurement reflect maximum ventricular filling when the heart was relaxed. Systolic dimensions were a reflector of systolic function in a heart, and not used to assess the presence or absence of dilation. The same principle applied to wall and septal thickness measurements. The presence or absence of hypertrophy were determined from
diastolic measurement of thickness. Systolic measurements of wall thickness were a reflection of systolic functions so reduced thickness during systole might simply reflect decreased function as reported by Boon, (2011).

Parameters of systolic function were always altered in DCM. A highly significant decrease in the fraction shortening were observed in DCM dogs compared to control. This findings are in agreement with Calvert et al. (1982); Atkins and Snyder (1992); Monnet et al. (1995); Tidholm and Jonsson (1996); Borgarelli et al. (1997); Calvert et al. (1997b); McEwan (1999); McEwan (2000); McEwan et al. (2003) and Borgarelli et al. (2006). Tidholom and Jonsson (1996) in a study of 37 Newfoundland dogs with DCM recorded left ventricular hypokinesis in 100 per cent of cases with FS ranging from 5 to 22 per cent which concurs with the present study.

In the present study there were a highly significant increase in EPSS in DCM dogs compared to control. This finding concurred with several authors (Calvert et al. 1982; Calvert and Brown 1986; Monnet et al. 1995; McEwan, 1999; Vollmar 1999; McEwan 2000 and Vollmar 2000). Calvert and Brown (1986) opined that EPSS were a most sensitive and specific criteria for the recognition of early cardiomyopathy. McEwan et al. (2003) proposed increased mitral valve EPSS as one among the guidelines for the diagnosis of DCM. Boon (2011) concluded that E-point to septal separation were one of the consistent (Normal value 7.7 mm) and popular mitral valve measurements. EPSS had strong negative correlation to ejection fraction in the absence of aortic and mitral insufficiencies. This correlation to ejection fraction were based on the fact that flow into the ventricle were equal to flow leaving ventricle. Therefore in DCM presence of high end diastolic left ventricular pressures reduced the flow from left atrium to left ventricle due to reduced ventricular compliance and consequently flow out of left ventricle were also reduced. EPSS accurately separated normal from abnormal left ventricular function regardless of left ventricular size when dilatation were present. Hence EPSS is also valid for assessing left ventricular function in the presence of abnormal septal motion as reported by above authors.
In Group III with MVD a highly significant increase in the mean values of FS; and a highly significant decrease in RVIDs, IVSs, LVIDd, LVIDs, LVPWd, LVPWs, EPSS; and a significant decrease in RVIDd, IVSFT and LVPWFT were noticed within groups. In MVD group left ventricular and left atrial dilation, exaggerated IVS and LVPW motion were the findings when comparing to standard reference ranges of Boon (2011). This findings are in accordance with Lombard et al. (1985) and Chetboul and Tissier (2012).

Boon (2011) reported that trace to mild MR seen in 10 per cent normal healthy dogs. Normal healthy dogs without any MVD will have FS of above 30 per cent. Dogs with any degree of mitral insufficiency, with no myocardial dysfunction, from mild to severe will have FS above the normal range called as supernormal FS. Kittleson et al. (1984) reported that MVD dogs without systolic failure will have FS above 60 per cent; and MVD dogs with severe systolic failure will have FS less than 60. In this study the FS of MVD group were 42.47±2.04 per cent and it might be due to moderate to severe systolic failure of myocardium. This concurs with Kittleson et al. (1984).

It was important to remember that fractional shortening was not a measure of contractility but it was a measure of function. The three conditions that affect the fraction shortening the most were preload (increased with exercise, increased blood volume, AV fistula, and excitement), after load (hypertension, aortic stenosis and insufficiency) and contractility as in DCM, MVD, pericardial effusion and HCM. Each one of these might individually or together affect the FS. When a low fractional shortening were calculated, it might be secondary to poor preload, increased after load or decreased contractility. To differentiate between these factors left ventricular end diastolic dimension measurement and blood pressure measurement were important. When increased left ventricular diastolic size were present with normal blood pressure, then reduced preload and increased afterload can be ruled out. Therefore measurement of blood pressure and LVIDd remains as a deciding factor to say that poor contractility is present. This is in agreement with Douglas and Tallant, (1991).
In Group IV with pericardial effusion a highly significant increase in the mean values of RVIDs; and a significant increase in RVIDd were noticed compared to control, where as a highly significant decrease in IVSs, LVIDd, LVIDs, LVPWd, LVPWs and FS; and a significant decrease in IVSFT and LVPWFT were noticed. This is in concurrence with Hoit (2007). Shaw and Rush (2007) concluded that pericardial effusion mostly ends in right side heart failure. In this study RV chamber dilatation were observed. This are in similarity with the above author.

In Group V with HCM a highly significant increase in IVSs, LVPWd, LVPWs and FS were observed. In addition a significant increase in IVSFT had noticed. A highly significant decrease in RVIDs, LVIDd, LVIDs; and a significant decrease in RVIDd and LVPWFT noticed in this group. This might be due to concentric LV hypertrophy, septum and/or wall. This results in impaired relaxation as reported by Atkins and Synder, 1991. Systolic function were generally not impaired in patients with HCM, so FS were normal or increased. In some cases systolic anterior motion (SAM) of MV could be observed which block the LV outflow which could lead to syncope as clinical sign in HCM dogs. This is in concurs with Kovacic and Muller (2003).

5.8.3 Colour flow doppler echocardiography (CFD)

In DCM affected dogs mitral valve regurgitation were noticed in 86.21 per cent of dogs and Tricuspid valve regurgitation were noticed in 34.48 per cent of dogs. Both mitral and tricuspid valve regurgitation were noticed in 31.03 per cent of dogs. Mild to severe form of Mitral insufficiency were noticed in DCM dogs which occurs secondary to dilation of the mitral annulus and improper closure of Mitral and Tricuspid valves which were the cause of murmur in some cases. Valvular lesions were not evidenced. The similar findings were observed by Kienle and Thomas (1995).

In MVD affected dogs mitral valve regurgitation were noticed in 100.00 per cent of cases. In this study mild MR in 16 per cent, moderate MR in 26 per cent and severe MR in 58 per cent were observed. Boon (2011) assessed regurgitation semi quantitatively by measuring the size of colour flow set within
the atria for atrio-ventricular (AV) valves. A lesion was classified as mild (<20% of the atrial chamber) if the insufficiency signal was detected only in the region close to the atrio-ventricular valves. A lesion was considered to be of moderate (20-60% of atrial chamber) severity if the signal was detected into the mid-region of the affected atrium. And finally, the insufficiency was classified as severe (>60% of atrial chamber) if the turbulent signal of atrio-ventricular valve insufficiency was detected at the base of the affected atrium or in the inlet vessels to the affected atrium. Darke et al. (1996) and Kittleson (1998) demonstrated the similar findings.

In the present study mild regurgitation in pericardial effusion were observed. This regurgitation might be due to abnormal distortion of the chambers leading to altered geometry and consequent leak in the AV valves as experienced by Tidholm et al., (2001).

In the present study all HCM cases showed normal aortic flow which indicated the primary nature of the HCM. The similar findings were observed by Thomas et al. (1984) and Fox (2003). The secondary HCM can occur due to aortic stenosis and hypertension as observed by Douglas and Tallant (1991) were not recorded in this study.

5.9 TISSUE DOPPLER IMAGING

Tissue Doppler Imaging provided information regarding myocardial velocity in selected areas of the myocardium. TDI evaluation of mitral annular motion on the left ventricular MA were less load dependant (Chetbol, 2002).

The pulsed wave TDI mode provided information on myocardial movements through a single sample volume in real time. This sample volume were placed at mitral annulus in left parasternal long axis view. This mode were very useful in analyzing the peak myocardial systole and diastolic velocity. With pulsed wave TDI mode, velocity analysis were limited to the specific region where the sample volume were placed. Mitral annulus were relatively static throughout the cardiac cycle.
The 2D color TDI mode gave the best “overall impression” of myocardial movements because all velocity data were displayed simultaneously on the screen. The tissue velocities towards the transducer were colored in red, and away from the transducer were colored in blue. No quantification parameters could be measured in 2D TDI.

With the color M-mode, real time myocardial velocity analysis were not possible. By comparison with 2D TDI imaging, M-mode TDI afforded a much higher temporal resolution. The different phases of the cardiac cycle were identified in real time.

Among the modes of TDI, PW-TDI were found to be superior in measuring the peak myocardial velocities than other TDI modes. PW-TDI provided the velocity information of myocardium from a single sample volume and it given systolic and diastolic velocity. Mitral annulus velocity were found to be good surrogate for PW-TDI because of its less load dependent nature in heart. All the three modes recorded are in agreement with Chetboul (2002).

5.9.1 Pulsed-wave Tissue Doppler Imaging (PW-TDI)

The Mean±S.E values of PW-TDI at mitral annulus in control and AHD dogs are given in Table-X.

In the present study a highly significant decrease in Sm and Em/Am ratio and a significant decrease in Em were noticed in all groups; and a significant increase in Am in pericardial effusion and HCM were observed. Peak systolic velocity (Sm) were highly significantly decreased in all groups. It proved that we can measure systolic failure with PW TDI. Similarly early diastolic (Em) velocity also decreased in all groups. Late diastolic velocity were increased in pericardial effusion and HCM. It was very typical finding with diastolic failure. It concluded that in DCM and MVD in addition to systolic failure, moderate diastolic failure were also present. Very peculiar Em/Am ratio reversal (Em/Am<1) were observed in pericardial effusion and HCM groups, and diastolic failure were more prominent when compared to DCM and MVD. Post systolic contraction velocity
could be observed in DCM and MVD. Hence it was concluded that both systolic
and diastolic failure can be recognised precisely with TDI. These findings are in
concurrence with findings of O’Sullivan et al., (2007), Chetboul (2002), and
Teshima et al. (2005).

Chetboul (2002) stated that diastolic dysfunction were the result of
impaired myocardial relaxation, restriction to LV filling and increased LV filling
pressures. Prolonged IVRT indicates poor myocardial relaxation. In diastolic
failure due to abnormal relaxation, IVRT were usually in excess. With restrictive
ventricular filling, it were usually lower. In this study pericardial effusion it were
lower than control and it indicates restrictive ventricular filling. In HCM group
there were statistically no significant changes observed in IVRT. But prolonged
IVRT were observed in this when compared to control. It was indicated the poor
myocardial relaxation. Moreover IVRT and IVCT values were mainly based on
the heart rate. In tachycardia the time taken for contraction and relaxation were
very less. IVRT and IVCT values has negative correlation with heart rate in this
study. Rapid heart rates limited the adequate LV filling and late diastolic phases
were coincide. Hence IVRT may not be reliable indicator in assessing the diastolic
failure in tachycardiac animals with restrictive LV filling as observed in this
study.

The LV filling pattern with HCM were abnormal (Kovacic and Muller,
2003). HCM group shows decreased early passive filling (Em) and a larger late
diastolic filling component (Am) with atrial contraction. This is in agreement with
the above authors.

Normally in healthy animals the passive filling component (Em) were
higher than atrial contraction (Am) component in diastole. Under normal
circumstances, the LV expands symmetrically during rapid, early filling. In
diastolic failure cases due to increased LV filling pressure, cardiac tamponade and
restrictive filling the Em were less than Am. In later stage of diastolic failure due
to increase in atrial pressure the Em becomes pseudonormal that is Em were more
than Am when assessing in normal Doppler echocardiography. In PW-TDI even
in pseudonormal cases the reversal of Em/Am (Em/Am<1) remains unaltered.
This were a classifical feature with TDI in diastolic failure cases. In this study Em/Am ratio reversal were observed in pericardial effusion and HCM. It showed that Em, Am, Em/Am indices were superior when compare the normal Doppler indices in diagnosing diastolic failure. These findigs are in similarity with Kibar et al. (2009); Tidholm et al. (2009); and Chetboul and Tissier (2012).

In this study PW TDI proved that it were very effective in quantifying regional systolic (Sm) and diastolic velocities (Em and Am) and Em/Am ratio more precisely and diagnosis of diastolic failure in AHDs as reported by Chetboul (2010).

A highly significant decrease were observed in all groups for global TDI velocity. Global velocity were the total velocity of myocardium in systole and diastole. It shows that all acquired heart diseases had decreased myocardial performance globally (Chetboul, 2002).

5.10 NT-proBNP MARKER ASSAY

The Mean±S.E values of NT proBNP assay in control and AHD dogs are given in Table-XI.

A highly significant increase were observed in Dilated Cardiomyopathy, MVD with systolic failure, MVD without systolic failure and Occult cardiac diseases.

Vanderheyden et al. (2004) reported that natriuretic peptides family contains of three major peptides, ANP, BNP, CNP, that participate in cardiovascular and cardiorenal homeostasis. Each of these natriuretic peptides binds differentially to specific receptors that signal through different mechanisms. Because of its fast induction and specific expression in cardiac diseases, BNP seems the most promising natriuretic peptide. It were predominantly synthesized in the cardiac ventricles, released as pre-proBNP and then enzymatically cleaved to BNP and the N-terminal portion of BNP(NT-proBNP). Blood measurements of BNP and NT-proBNP had shown to identify patients with LV dysfunction.

Streching of ventricular myocardium results in release of NT-proBNP into the circulation (Oyama et al., 2009). NT-proBNP were used for screening and
prognosis of heart failure (Bhalla et al., 2004). NT-proBNP typically increased in dogs with left ventricular dysfunction, with or without symptoms. In this study based on the levels of NT-proBNP the severity of AHDs were assessed. This shows that NT-proBNP were an effective and specific marker in diagnosis and categorising the cardiac diseases even in occult form. These findings are in concurrence with Oyama and Singletary, (2010); Oyama et al. (2009); Serres et al. (2009); Moonamart et al. (2010); Kellihan et al. (2011) and Ettinger et al. (2012).

In this study even though NT-proBNP were found to be effective and specific marker in cardiac disease diagnosis, it had certain limitations like sample size required, cost of the test involved and controlled lab setting.

5.11 POST-MORTEM FINDINGS

5.11.1 Gross pathology

In DCM heart were enlarged, globular, flabby in appearance with rounded apex due to generalized ventricular dilatation. On transverse biventricular slice of heart marked dilatation of lumen, attenuation of papillary muscles and thinning of free walls of ventricles were observed. These findings were in agreement with (Sisson and Thomas, 1995; Tidholm and Jonsson, 1997). They reported dilation of all four cardiac chambers or predominant dilation of left chambers. Myocardial eccentricity were evident with decreased ratio of left ventricular wall to chamber diameter as reported by several authors (Sisson and Thomas 1995; Tidholm and Jonsson, 1996; Sisson et al., 1999)

5.10.2 Histopathology

In DCM the histopathology of ventricular myocardium, attenuated way fibre type of myofibres with thinner, well separated and wavy appearance were identified in four dogs. In addition, focal chronic myocarditis and necrosis were present with infiltration of collagen fibrinous materials into the cardiac muscle fibres. Tilley and Liu, (1975); Tidholm et al. (1998) and Dambach et al. (1999) reported attenuated wavy fibre as the major histological findings in dogs with DCM which concurs with the present findings.
CHAPTER VI
SUMMARY AND CONCLUSIONS

The study entitled “Doppler echocardiographic evaluation of acquired heart diseases in dogs” was carried out at the Centre of Advanced Faculty Training in Veterinary Clinical Medicine, Ethics and Jurisprudence, Madras Veterinary College, TANUVAS, Chennai-7 during the period February, 2012 - February, 2014, with the following objectives,

- To quantify the global and regional myocardial function by Tissue Doppler echocardiography in acquired heart diseases of dogs
- To compare the diagnostic efficacy of different modes of Tissue Doppler echocardiography in dogs
- To establish the usefulness of biochemical marker in diagnosis of acquired heart diseases in dogs

The study was conducted with the clinical cases presented to the Small Animal Medical Outpatient Clinic of the Madras Veterinary College Teaching Hospital over a period of five semesters. The Clinical study comprised of both healthy dogs and dogs with Acquired Heart Diseases (AHD) and they were grouped as following:

- **Group I:** Apparently healthy dogs (Control group)
- **Group II:** Dilated Cardiomyopathy (DCM)
- **Group III:** Mitral Valve Disease (MVD)
- **Group IV:** Pericardial effusion
- **Group V:** Hypertrophic Cardiomyopathy (HCM)

Parameters of the study included Prevalence analysis, Medical history, Clinical presentation, Baseline Haematology panel, Baseline serum biochemistry panel, Radiography, Doppler BP, ECG, Echocardiographic indices such as 2-D echocardiographic indices, M-Mode Echocardiographic indices and Colour flow Doppler findings, Pulsed Wave Tissue Doppler Imaging, NT-proBNP Assay and Post-mortem examination.

The incidence of AHDs were found to be 0.37 per cent (106 out of 28467) in
the five semester study period. Labradors and Spitz were found to be commonly affected with DCM and MVD respectively. Older dogs were found to be more commonly affected with AHDs and the incidence were higher in 8-10 years of age group (23.58 per cent) followed by 6-8 years of age group (19.81 per cent). Males (75 per cent) were found to be more commonly affected than females (25 per cent). The observed chief complaints included inappetance, exercise intolerance, abdominal enlargement, syncope and weakness. Tachycardia, ascites and murmurs were the common physical examination findings in all the groups of AHDs. Presence of signs such as Tachycardia (86.21 per cent) and ascites (82.76 per cent) in DCM; and coughing (89.74 per cent) and murmur (89.74 per cent) in MVD showed the presence of acquired heart disease in dogs.

Haematological assessment showed no significant changes. Serum biochemical assessment showed a significant hypernatremia in all groups except HCM. Otherwise no significant changes in the routinely assessed biochemical parameters in dogs with AHDs.

Radiographic signs of AHDs included cardiomegaly, pulmonary oedema and left atrial enlargement. Dogs with DCM and pericardial effusion had significantly elevated VHS and confirmed the presence of acquired heart disease.

ECG findings in AHDs were the DCM dogs had characteristic arrhythmic pattern of atrial fibrillation in 20.69 per cent cases and atrial flutter in 8.62 per cent of cases. MVD group dogs had atrial enlargement in 51.28 per cent; pericardial effusion group had low voltage QRS complex and electrical alternans in 83.33 per cent; and HCM group had ventricular enlargement in 100.00 per cent cases.

In two dimensional echocardiography a highly significant increase in the mean±S.E values of LA, LA/Ao, Simpson EDV, Simpson ESV and a highly significant decrease in the Simpson EF were noticed in DCM. A highly significant increase in the mean±S.E values of LA and LA/Ao were noticed in MVD and Pericardial Effusion were observed. In HCM a highly significant decrease in LA and increase in LA/Ao were observed.
In M-mode echocardiography a highly significant increase in LVIDd, LVIDs and EPSS of DCM group, FS of MVD and HCM group, LVPWd of HCM group; and a highly significant decrease in FS in DCM group; and LVIDd & LVIDs of HCM group were the classical and important findings. In MVD supernormal FS (>60 per cent) were a normal finding in animals without heart failure. The FS <60 per cent indicates systolic failure.

In DCM dogs no change in the flow pattern were observed across Ao and PA in any of the dogs, where as normal flow pattern across MV and TV were observed in only 13.79 per cent of dogs. Mitral valve regurgitation were noticed in 86.21 per cent of dogs and Tricuspid valve regurgitation were noticed in 34.48 per cent of dogs. Both mitral and tricuspid valve regurgitation were noticed in 31.03 per cent of dogs. In MVD dogs mitral valve regurgitation were noticed in 100.00 per cent of dogs with normal flow pattern in Aorta and Pulmonary Artery. Mitral regurgitation in pericardial effusion might be due to altered geometry of LV.

In concise, DCM dogs had LV dilatation, LA dilatation, increased LA/Ao ratio, decreased LVFW and septal thickness, decreased FS, increased LVIDs & LVIDd, increased EPSS and secondary regurgitation of mitral valve and Tricuspid valve were identified with Doppler Echocardiography. In MVD dogs LA dilatation, increased LA/Ao ratio, FS <60 per cent were identified. Colour flow Doppler were useful in qualitatively categorising the regurgitation as mild, moderate and severe. In pericardial effusion cases echo-free space around ventricular chambers, Right Atrial collapse indicating tamponade were present in Doppler echocardiography. In HCM dogs LV hypertrophy, SAM and increased FS were identified.

In pulsed wave tissue Doppler a highly significant decrease in Sm and Em/Am ratio all groups; and significant decrease in Em in all groups were observed; and significant increase in Am in pericardial effusion and HCM were observed. Very typical Em/Am ratio reversal (Em/Am<1) were observed in pericardial effusion and HCM group.

In brief, Pulsed wave TDI were very much useful in assessing the peak myocardial velocities at mitral annulus and in diagnosing the diastolic failure.
Pulsed wave TDI mode were useful in assessing the global velocity of myocardium. In DCM and MVD dogs decreased Sm at lateral mitral annulus, decreased Em, decreased global velocity were observed, indicating systolic failure and mild diastolic filling defect. In pericardial effusion decreased Em, Em/Am reversal (Em/Am<1), restrictive filling pattern, decreased global velocity and severe diastolic failure were observed. In HCM dogs decreased Em, Em/Am reversal (Em/Am<1), decreased Sm, decreased global velocity, impaired relaxation and severe diastolic failure were observed.

In NT-proBNP assay a highly significant increase in levels were observed in Dilated Cardiomyopathy, MVD with systolic failure, MVD without systolic failure and even in occult cardiac diseases which indicated that this marker is a highly sensitive and specific in cardiac diseases. But the major limitation of this test it requires bigger sample size.

Post-mortem examination were performed only in 4 dogs with DCM during the study period. Grossly the heart were enlarged, globular, and flabby in appearance with rounded apex due to generalized ventricular dilatation. On transverse biventricular slice of heart the marked dilatation of lumen, attenuation of papillary muscles and thinning of free walls of ventricles were observed. On histopathology left ventricular myocardium showed attenuated wavy fibre type of changes.

CONCLUSIONS

From the above study the following conclusions were made:

1. In Tissue Doppler Echocardiography of acquired heart diseases the regional and global velocities were highly significantly decreased in dogs. Tissue Doppler Imaging was a highly effective in diagnosing diastolic failures even at occult stage. Regarding systolic failure it can be added an aid in diagnosing the condition.

2. Among the modes Pulsed wave Tissue Doppler Echocardiography is very effective in assessing the peak systolic and diastolic velocities in real time, when compared to other modes in dogs.
The cardiac biomarker NT-proBNP was very effective and specific in diagnosing and staging the cardiac diseases in dogs even at occult stage.
REFERENCES


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**ANNEXURE-I**

**DOPPLER ECHOCARDIOGRAPHIC ASSESSMENT OF ACQUIRED HEART DISEASES IN DOGS**

Canine Clinical Case Proforma

<table>
<thead>
<tr>
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**Presenting Problem:**

**Case summary:**

**Prior Treatment/Medications:**

**Vitals:**

<table>
<thead>
<tr>
<th>Temp:</th>
<th>Exercise intolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate:</td>
<td>Dyspnoea and Tachypnoea</td>
</tr>
<tr>
<td>Pulse rate:</td>
<td>Edema/Ascites:</td>
</tr>
<tr>
<td>Respiratory Rate:</td>
<td>Cyanosis</td>
</tr>
<tr>
<td>CMM:</td>
<td>Syncope</td>
</tr>
<tr>
<td>CRT:</td>
<td>Cough</td>
</tr>
</tbody>
</table>

**Auscultation of Heart:**

<table>
<thead>
<tr>
<th>Rate:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhythm</td>
</tr>
<tr>
<td>Amplitude</td>
</tr>
<tr>
<td>Murmer</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

**Haematology and Biochemistry**

| Hb |  |
| PCV | BUN |
| RBC | Creatinine |
| WBC | Total Protein |
| Platelets | Albumin |

<table>
<thead>
<tr>
<th>Differential count</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>ALP</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Calcium</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>Phosphorus</td>
</tr>
<tr>
<td>-------------</td>
<td>------------</td>
</tr>
<tr>
<td>Monocytes</td>
<td>Sodium</td>
</tr>
<tr>
<td>Basophils</td>
<td>Potassium</td>
</tr>
</tbody>
</table>

**Systolic BP(Doppler):**

**ECG:**

<table>
<thead>
<tr>
<th>HR:</th>
<th>No:</th>
<th>Date:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impression</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Atrial and/or ventricular enlargement patterns
- Tachyarrhythmias
- Atrial fibrillation
- Ventricular tachyarrhythmias
- Sinus tachycardia

**X-Ray:**

<table>
<thead>
<tr>
<th>No:</th>
<th>Date:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impression</td>
<td></td>
</tr>
</tbody>
</table>

- Within normal limits
- Atrial and ventricular enlargement (left)
- Pulmonary venous distension
- Pulmonary oedema

**Echo Cardiography:**

<table>
<thead>
<tr>
<th>Aorta</th>
<th>RVIDd</th>
<th>EDV</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA</td>
<td>RVIDs</td>
<td>ESV</td>
</tr>
<tr>
<td>LA/Ao</td>
<td>IVSd</td>
<td>SV</td>
</tr>
<tr>
<td>LVIDd:</td>
<td>IVSs</td>
<td>EF</td>
</tr>
<tr>
<td>LVIDs:</td>
<td>LVFWd</td>
<td></td>
</tr>
<tr>
<td>FS:</td>
<td>LVFWs</td>
<td></td>
</tr>
</tbody>
</table>

**Tissue Doppler Imaging (TDI):**

**TDI variables**

<table>
<thead>
<tr>
<th>MA</th>
<th>IVS</th>
<th>LVFW</th>
</tr>
</thead>
</table>

- Sm - systolic myocardial velocity:
- Em - early diastolic myocardial relaxation velocity
- Am - myocardial velocity associated with atrial contraction.
- Em/Am ratio
- IVRT – Isovolumic Relaxation Time
- IVCT - Isovolumic Contraction Time
- Global TDI = S x E/A

**Diagnosis:**

**Biomarkers:** NT proBNP - ELISA