EVALUATION OF CARDIAC BIOMARKERS AND ECHOCARDIOGRAPHY IN THE DIAGNOSIS AND PROGNOSTICATION OF MITRAL VALVE DISEASE AND DILATED CARDIOMYOPATHY IN DOGS

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Certificate

This is to certify that the thesis entitled "***Evaluation of cardiac biomarkers and echocardiography in the diagnosis and prognostication of mitral valve disease and dilated cardiomyopathy in dogs****"* submitted by **MS. DEEPTI B. R**., **I.D.No. DVHK 1215** in partial fulfilment of the requirements for the award of degree of **DOCTOR OF PHILOSOPHY** in **VETERINARY MEDICINE** of the Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar is a record of bonafide research work carried out by her during the period of her study in this University under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associationship, fellowship or other similar titles.

Place: Bangalore
Date: May, 2015

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Once again,

to those who taught me

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%: per cent

˚ C: degree Celsius

<: less than

>: more than

≤: less than or equal to

≥: more than or equal to

µL: microlitre

2D: two dimensional

ACE: angiotensin converting enzyme

AHA: American Heart Association

ALT: alanine amino transferase

ANP: atrial natriuretic peptide

Ao: aorta

ARJ/LAA: area of regurgitant jet/ left atrial area

ATII: angiotensin II

BNP: brain/ B-type natriuretic peptide

bpm: beats per minute

BW: body weight

cap: capsule

CHF: congestive heart failure

CHIEF: Canine Heart failure International Expert Forum

CK-MB: creatine kinase myocardial band

cm: centimetre

CMVD: chronic mitral valvular disease

CT: computed tomography

cTnC: cardiac troponin C

cTnI: cardiac troponin I

cTnT: cardiac troponin T

DCM: dilated cardiomyopathy

DMVD: degenerative mitral valve disease

DNP: dendroaspis natriuretic peptide

ECG: electrocardiogram

EDTA: ethylene diamine tetraacetic acid

EDV: end-diastolic volume

EF: ejection fraction

EPSS: E-point to septal separation

ESV: end-systolic volume

FS: fractional shortening

g: gram

HCl: hydrochloride

HF: heart failure

i.e.: that is

ISACHC: International Small Animal Cardiac Health Council

IU/L: international units per litre

IVS_d: interventricular septum in diastole

$IVS_{s:}$ interventricular septum in systole

kg: kilogram

LA/Ao: left atrium to aorta ratio

LA: left atrium

LDH: lactate dehydrogenase

LV: left ventricle

$LVFW_{d:}$ left ventricular free wall (thickness) in diatole

$LVFW_s$: left ventricular free wall (thickness) in systole

$LVID_d$: left ventricular internal diameter in diastole

$LVID_s$: left ventricular internal diameter in systole

mA: milli ampere

mg/kg: milligram per kilogram

mg/mL: milligram per millilitre

Mhz: megahertz

MI: myocardial infarction

mL: millilitre

mm/s: millimetre per second

mm: millimetre

M-mode: motion mode

m^2: metre square

MMVD: myxomatous mitral valve disease

MR: mitral regurgitation

MVD: mitral valve disease

OD: optical density

NEP: neutral endopeptidase

ng/mL: nanogram per millilitre

NP: natriuretic peptide

NPR: natriuretic peptide receptor

NT-pro BNP: amino terminal pro-B-type natriuretic peptide

NYHA: New York Heart Association

pmol/L: picomoles per litre

Pvt. Ltd: private limited

q8-12 h: once in 8-12 hours

RAAS: renin angiotensin aldosterone system

rpm: revolutions per minute

SF: shortening fraction

tsp: tea spoon

viz: namely

VNP: ventricular natriuretic peptide

WHO: World Health Organization

INTRODUCTION

I. INTRODUCTION

Cardiac diseases are common in dogs and up to 10% of the cases presented to primary care veterinary practices are those of heart diseases. They are a significant cause of morbidity and mortality. Heart failure is a syndrome with signs caused by cardiac dysfunction, resulting in reduced longevity and is generally a chronic condition. It has become a major and growing public health problem and appears to result not only from cardiac overload or injury but also from a complex interplay among genetic, neurohormonal, inflammatory, and biochemical changes acting on cardiac myocytes, the cardiac interstitium, or both (Braunwald, 2008). To support the failing heart, numerous compensatory mechanisms occur, including activation of the neurohormonal system (Mosterd and Hoes, 2007).

The two most common causes of heart failure in the dog are mitral regurgitation (MR) due to acquired valvular disease (chronic mitral valvular disease or endocardiosis) and myocardial failure due to dilated cardiomyopathy (DCM) (Woodfield *et al.*, 1995a).

Increased life expectancy in dogs due to the advances in canine medicine and surgery as well as preventive medicine has led to cardiac diseases becoming very common in the geriatric dog and in one study was the second most common cause of death (Guglielmini, 2003).

Small animal clinicians are accustomed to diagnosing and managing advanced stages of heart disease with overt signs of congestive heart failure; however, the diagnosis and management of earlier stages of heart disease and occult heart disease is more

challenging (Collins, 2013). A simple and inexpensive diagnostic test, with high sensitivity and specificity, which could provide the small animal general practitioner with reliable diagnostic and prognostic information about heart disease, would be a useful tool.

Circulating markers of heart disease in blood (cardiac biomarkers) have shown promise for this purpose and have been the subject of considerable interest in the veterinary literature (Reynolds and Oyama, 2008); however, their utility remains largely untested outside research establishments. Other advantages of such a biomarker would be because of the prospect of identifying asymptomatic heart disease, early diagnosis, identification of heart disease without extensive training in cardiology, clarification of the status of dogs with equivocal results by other diagnostic methods (Sisson, 2000), estimation of the risk of onset of congestive heart failure in dogs (Reynolds *et al.,* 2012), independent prognostic factor for the progression of heart failure (Ebisawa *et al.,* 2012), differentiating between heart or respiratory disease in a coughing or dyspnoeic dog (DeFrancesco *et al.,* 2007), predicting survival times (Fonfara *et al*., 2010) and outcome in dogs with congestive heart failure (Linklater *et al*., 2007), monitoring and guiding therapy (Wolf *et al*., 2012) and even using some variant of the biologically active molecule (in case of B-type natriuretic peptides) to treat patients with heart diseases (Sisson, 2009).

Evaluation of heart function is usually accomplished by electrocardiography, radiography and where available by echocardiography. Diagnosis is difficult in animals with respiratory distress as they may not tolerate the imaging techniques and ECG may not be of diagnostic importance in the absence of arrhythmias. Radiography has traditionally been used for the diagnosis of congestive heart failure (CHF) caused by various cardiac

diseases but it is not always possible to identify the etiology of CHF by this method and this method can suffer from considerable observer variation (Schober *et al*., 2010).

Confirmation of the cause of CHF requires echocardiography (Boswood, 2008). Ultrasound examination of the heart can identify valve lesions, and confirm presence of regurgitation in mitral valve disease (MVD) and influence prognosis and selection of medical therapy (Bonagura and Schober, 2009). Echocardiographic findings in MVD include valve thickening and abnormal motion of the valve leaflets and the left atrium to aorta ratio is the most significant independent predictor of mortality (Borgarelli *et al*., 2008). In DCM, left ventricular dimensions, geometry and functional indices like fractional shortening are affected (Stephenson *et al.*, 2012).

The mainstay of therapy for CHF caused by MVD has been diuretics with or without digoxin. Angiotensin converting enzyme (ACE) inhibitor enalapril was successful in increasing survival times in patients when combined with the standard treatment (Ettinger *et al*., 1998) and ACE inhibitor benazepril was shown to have a similar effect (Pouchelon *et al.,* 1999). The conventional therapy for CHF due to DCM is also by use of diuretics, ACE inhibitors and positive inotropes (Soares *et al*., 2010). Despite improvements in pharmacological treatment and prevention, heart failure remains a serious problem, and carries a poor prognosis. Newer medications now available to the veterinary cardiologists include inodilators like pimobendan and nutraceuticals which show promise in the management of CHF.

There is paucity of information regarding normal biomarker levels in dogs and their values in heart disease and CHF in Indian literature. Also not much importance has been given to the prognostic potential of various tests. So the present study was undertaken with the following objectives:

1. To study the occurrence of mitral valve disease and dilated cardiomyopathy in dogs.
2. To study the levels of cardiac biomarkers in mitral valve disease and dilated cardiomyopathy of dogs and compare human and canine troponin kits.
3. To study the changes associated with mitral valve disease and cardiomyopathy in dogs using echocardiography.
4. To assess the prognostic value of cardiac biomarkers and echocardiographic findings by follow up of cases during the treatment period.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

The available literature have been reviewed under the following headings

2.1 DEFINITIONS

The definitions of the terms used in the title of the thesis have been given below, so as to avoid any ambiguity.

2.1.1 Dilated Cardiomyopathy

Dilated cardiomyopathy (DCM) is the dilatation and impaired contraction of the left or both ventricles. The diagnosis of idiopathic DCM requires the active exclusion of other cardiac, pulmonary or systemic diseases which may secondarily induce a similar phenotype (Dukes-McEwan *et al.*, 2003). DCM is a primary myocardial disease characterised by cardiac enlargement and impaired systolic function of one or both ventricles (Meurs, 2010).

2.1.2 Mitral Valve Disease

Mitral valve disease (MVD), also called myxomatous mitral valve disease (MMVD) or mitral endocardiosis or chronic mitral valvular disease (CMVD) is usually caused by a progressive myxomatous degeneration of the mitral valve (Olsen *et al*., 2010).

Mitral valve disease (MVD) is characterized by chronic myxomatous mitral valve degeneration resulting in thickening and incomplete apposition of the valve leaflets during systole with secondary mitral valve regurgitation (Chetboul and Tissier, 2012).

2.1.3 Biomarker

The term “biomarker” or “biological marker” was introduced in 1989 (Vasan, 2006) and refers to a broad subcategory of medical signs – that is, objective indications of medical state observed from outside the patient – which can be measured accurately and reproducibly (Strimbu and Tavel, 2010).

World Health Organization (WHO) has stated that a true definition of biomarkers includes “almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, or biological. The measured response may be functional and physiological, biochemical at the cellular level, or a molecular interaction.” (WHO, 1993).

The International Programme on Chemical Safety, led by the WHO and in coordination with the United Nations and the International Labour Organization, has defined a biomarker as “any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease” (WHO, 2001).

National Institutes of Health Biomarkers Definitions Working Group defined a biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” (Atkinson *et al*., 2001).

A biomarker may be measured on a biosample (as a blood, urine, or tissue test), it may be a recording obtained from a person (blood pressure, ECG, or Holter), or it may be an imaging test (echocardiogram or CT scan) (Vasan, 2006). Although biomarkers include genetic variants, clinical images, physiological tests, and tissue specimen biopsies

(Braunwald, 2008), cardiac biomarkers in the conventional sense refers to substances measured in the blood (Boswood, 2009).

Biomarkers can be classified as antecedent biomarkers (identifying the risk of developing an illness), screening biomarkers (screening for subclinical disease), diagnostic biomarkers (recognizing overt disease), staging biomarkers (categorizing disease severity), or prognostic biomarkers (predicting future disease course, including recurrence and response to therapy, and monitoring efficacy of therapy) (Vasan, 2006).

2.2 EPIDEMIOLOGY OF HEART DISEASE

Although the true prevalence of heart disease in dogs is unknown, two independent studies completed 30 years apart suggest that approximately 11% of canine patients presenting for evaluation at large veterinary hospitals have cardiac disease (Parker *et al*., 2006).

2.2.1 Mitral valve disease

Mitral insufficiency resulting from chronic mitral valve fibrosis is the form of heart disease most frequently diagnosed in clinical practice (Ettinger and Suter, 1970).

Heart disease was the second most common cause of death in a survey in 1990s. MVD was the most common cause of heart disease (Linklater *et al*., 2007; Wolf *et al*., 2012), accounting for 75-80% of cardiac diseases in dogs (Borgarelli *et al*., 2008; Olsen *et al*., 2010).

Chronic degenerative valvular disease (CDVD) is the most common acquired heart disease in dogs - accounting for approximately 75% of all cases of congestive heart failure in dogs (James, 2009). The prevalence of MMVD is strongly age dependent, with few per cent in young dogs to approximately 75% in dogs above 16 years of age (Olsen *et al*., 2010). Its prevalence is higher in smaller dogs (< 20 kgs), although large breeds are occasionally affected and the disease is approximately 1.5 times more common in male dogs than in female dogs. Cavalier King Charles Spaniels are predisposed to developing MVD at a relatively young age (Atkins *et al*., 2009).

Degenerative mitral valve disease (DMVD) is the most common cardiac disease in dogs (Serres *et al*., 2009).

Ouellet *et al*. (2009) reported that MVD represents the underlying condition of the vast majority of cases presented to their hospital in Montreal, Canada as congestive heart failure (CHF)

The breeds most commonly affected with MVD are dogs of small to medium size like the Papillon, Poodle, Chihuahua, Dachshund and Cavalier King Charles Spaniel (Olsen *et al*., 2010; Parker and Kilroy-Glynn, 2012).

MVD is present in more than one-third of dogs over ten years of age (Reynolds *et al*., 2012; Wolf *et al*., 2012)

Prevalence of MVD is 14-40% in small-sized dogs and higher in geriatric dogs (Chetboul and Tissier, 2012).

2.2.2 Dilated cardiomyopathy

Idiopathic dilated cardiomyopathy occurs commonly in large dog breeds and males of the breeds (Ogburn, 1977; Lombard, 1984).

In a study by Sisson, (2000) the prevalence was 0.16% in mixed breeds where as it was 0.65% in purebred dogs. The median age of dogs with DCM was between 4 to 8 years, a generally younger population of dogs than those afflicted with degenerative valvular disease. Also in contrast to acquired valvular disease, there were a significant number of puppies younger than 1 year of age afflicted with DCM. Males were nearly twice as often affected than females with respect to development of heart failure or sudden death.

A male predominance has been reported in several studies and most dogs are initially presented at the age of 5 to 7 years (Tidholm *et al*., 2001).

Age of onset of congestive heart failure due to DCM ranged from 3.5 to 13 years with a mean of 6.6 years (Sleeper *et al*., 2002).

The most commonly reported inherited adult onset canine heart disease is DCM (Meurs, 2003). DCM is also the most common form of myocardial disease in the dog (Meurs, 2010).

The overall prevalence of DCM was 0.5% according to Purdue University records. Breed prevalence was higher in pedigree dogs with male dogs showing an early onset. DCM is a major cause of morbidity and mortality in various dog breeds (Dukes-McEwan *et al*., 2003).

A high incidence of cardiomyopathy is seen in Doberman Pinschers, Boxers, and giant breeds (Oyama and Sisson, 2004).

The Doberman Pinscher is one of the most common breeds of dogs to develop dilated cardiomyopathy, the disease is adult onset (median of 7.5 years at diagnosis) and appears particularly aggressive (Meurs *et al*., 2007). In Newfoundland, the median age of onset of clinical signs is approximately 8 years of age and the median age of death 9 years (Wiersma *et al*., 2008).

Generally DCM is a disease of large and medium sized dog breeds. Increased incidence is found in breeds like Doberman Pinscher, Irish Wolfhound, Great Dane, Cocker Spaniel and Newfoundland (Meurs, 2010). It is an adult onset disease with the exception of Portuguese water dog where it is diagnosed between 2-32 weeks.

Dilated cardiomyopathy (DCM) is the second most common cause of cardiac morbidity and mortality in dogs, mainly among large and giant breeds (Soares *et al*., 2010).

Doberman Pinschers at an age of 6 to 8 years showed a prevalence of 43.6% and older dogs a prevalence of 44.1%. The cumulative prevalence of DCM in Doberman Pinschers in Europe was demonstrated to be 58.2% (Steudemann *et al*., 2013).

2.3 BIOMARKERS

2.3.1 Characteristics and utility of an ideal biomarker

Vasan (2006) regards biomarkers as a tool to better identify high-risk individuals, to diagnose disease conditions promptly and accurately, and to effectively prognosticate and treat patients with disease.

According to Braunwald (2008), characteristics of an ideal biomarker includes accurate, repeated measurements available to the clinician at a reasonable cost and with

short turnaround times, provides information that is not already available from a careful clinical assessment and knowing the measured level aids in medical decision making.

Biomarker is a substance elaborated by a specific tissue that can be detected in circulation. An ideal biomarker is released in proportion to a particular disease process, and provides information regarding presence, severity, and prognosis of the disease. It must be stable and easy to detect with a widely available, rapid, and inexpensive assay. Traditionally, the evaluation of heart function has been accomplished by electrocardiography, radiography, and echocardiography. These tests are relatively time-consuming and expensive, and in the case of echocardiography, may not be available to all patients. (Reynolds and Oyama, 2008).

Biomarkers should aid in the diagnosis of heart failure, should be useful for screening patients at risk for heart disease and for monitoring and guiding therapy. Some variant of the biologically active molecule may even be useful to treat patients with heart disease (Sisson, 2009).

Another important characteristic of the optimal biomarker is that it should be independent from other factors (Ciccone *et al*., 2013).

2.3.2 Cardiac biomarkers of clinical utility

2.3.2.1 B-type Natriuretic Peptide

In 1981, atrial natriuretic peptide was discovered when injection of atrial tissue extracts in rats induced natriuresis (deBold *et al*., 1981). This lead to the discovery of a new class of hormones, the natriuretic peptides. These are the atrial natriuretic peptide, ANP; urodilatin (very similar to ANP, but produced by the kidneys); B-type natriuretic

peptide (BNP), C-type natriuretic peptide in 4 different isoforms, dendroaspis natriuretic peptide (DNP) and ventricular natriuretic peptide (VNP). ANP, BNP and VNP are primarily produced by cardiomyocytes. All NPs are synthesized as pre-pro-hormones. The actions of the NPs are mediated by a set of specific natriuretic peptide receptors (NPR), and variations in the effects of the different NPs primarily depend on the differences in local expression and production. Cardiac NPs decrease the renin secretion from the macula densa and inhibit aldosterone release from the zona glomerulosa and the angiotensin II-stimulated proximal tubular sodium and water reabsorption (Kimmenade and Januzzi, 2009).

B-type natriuretic peptide (BNP) is a cardiac peptide hormone originally isolated from porcine brain (Sudoh *et al*., 1988) and initially called the brain natriuretic peptide. BNP, now called B-type natriuretic peptide is found wherever myocardial tissue is present but particularly in the ventricles. BNP is rapidly produced by cardiomyocytes after stimuli like myocardial stretch or hypoxia (Kimmenade and Januzzi, 2009).

Production of BNP is rapidly up-regulated when cardiomyocytes are stimulated. BNP and its inactive amino-terminal fragment NT- proBNP are released in response to volume expansion or pressure overload (Schmidt *et al*., 2009). The most important stimuli are cardiomyocytal stretch and ischemia/hypoxia, but other stimuli such as endothelin-1, angiotensin-II, interleukin-1b and adrenergic agonists, also result in the production of the prepro-hormone, namely pre-proBNP$_{1\text{-}134}$ (Prosek and Ettinger, 2010). This is later split to produce the 108-amino acid propeptide, proBNP$_{1\text{-}108}$. The biologically inert 76-amino acid

amino-terminal part is NT-proBNP$_{1-76}$, and the biologically active 32-amino acid molecule BNP$_{1-32}$ (Reynolds and Oyama, 2008; Kimmenade and Januzzi, 2009).

The kidney, vasculature and the heart itself are target organs for BNP$_{1–32}$ (Baerts *et al*., 2012). It exerts its effect through natriuretic peptide receptors (NPRs). NPR-A stimulation results in natriuresis, inhibition of renin and aldosterone, as well as vasorelaxant, anti-fibrotic, anti-hypertrophic and lusitropic effects. Although stimulation of the NPR-B may not lead to natriuresis or diuresis, recent studies suggest that the NPR-B may play a more important cardioprotective role as was assumed up till now. NPR-C functions mainly as a modulator of NP availability at target organs. Some studies indicate that the NPR-C may mediate anti-proliferative effects of BNP and CNP in cardiac fibroblasts (Kimmenade and Januzzi, 2009).

BNP may also inhibit the cardiac sympathetic nervous system and renin-angiotensin-aldosterone system by suppressing norepinephrine and aldosterone levels, hence regulate blood volume and pressure and induce bronchoconstriction (Prosek and Ettinger, 2010).

Neutral endopeptidase (NEP), also called neprilysin, is a membrane-bound metalloprotease responsible for the active elimination from the circulation of NPs via hydrolysis. Amino-terminal natriuretic peptide pro-fragments, such as NT-proBNP, are not cleared by either NPRs or NEPs, and are thought to be cleared by organs with high degrees of blood flow, such as the kidney (Kimmenade and Januzzi, 2009). The half life of BNP is 90 seconds (Prosek and Ettinger, 2010). NT pro-BNP is, at present, used as a surrogate

marker for the biologically active form BNP because the peptide is more stable in vivo than the active form (Kellihan *et al*., 2011; Fukumoto *et al*., 2014).

Unfortunately, the diuretic and natriuretic properties of BNP in pathophysiological concentrations are still insufficient to retain euvolemia (Kimmenade and Januzzi, 2009; Baerts *et al*., 2012). BNP analogues with a higher affinity for NPRA and/or resistant to specific peptidases, as well as inhibitors of peptidases are promising investigational drugs for HF (Baerts *et al*., 2012).

NT-proBNP has also been shown to be the most powerful predictor of left ventricular dysfunction, risk stratification, and prognosis in humans with acute and chronic congestive heart failure. NT-proBNP concentration is an independent risk factor for morbidity and mortality in humans with DMVD (Serres *et al*., 2009).

BNP and NT-proBNP are significantly elevated in dogs with DMVD. They have shown potential for assessing heart disease in dogs. Plasma NT-proBNP concentration independently estimates risk of first-onset of CHF in dogs with DMVD (Reynolds *et al*., 2012).

Dogs with moderate to severe mitral regurgitation had significantly higher NT-proBNP than healthy dogs and those with mild MR (Chetboul and Tissier, 2012). More significantly higher values are obtained in dogs with severe MVD (Trafny *et al*., 2012).

NT-proBNP has also been shown to be the most powerful predictor of left ventricular dysfunction, risk stratification, and prognosis in humans with acute and chronic

congestive heart failure (Serres *et al*., 2009). BNP and NT-proBNP are significantly elevated in dogs with degenerative mitral valve disease (DMVD) and has shown potential for assessing heart disease in dogs. Brain natriuretic peptide (BNP) and NT-proBNP are the best known markers of heart failure (Ciccone *et al*., 2013).

NT-proBNP concentrations in normal individuals exhibited a wide range of values, suggesting inter-individual variability, with females having higher median plasma NT-proBNP values than males. Significant breed effect for plasma NTproBNP has also been found (Misbach *et al*., 2013). NT-proBNP increased significantly in dogs with renal dysfunction but free of cardiovascular disease (Schmidt *et al*., 2009). Reduced GFR and hypertension of chronic kidney disease may elevate NT-proBNP in animals without heart disease (Miyagawa *et al*., 2013). The natriuretic peptides are cleared by the kidneys, and the hypervolemia and hypertension characteristic of renal failure enhance the secretion and elevate the levels of BNP, especially the NT-pro-BNP (Braunwald, 2008).

In a study by Reynolds and Oyama (2008), a cut-off value of 210 pmol/L gave a positive predictive value of 94% and negative predictive value of 74%. This means that dogs with a positive test were 94% likely to have heart disease or failure while dogs with a negative test were 77% likely to not have heart disease or heart failure. A positive predictive value of 97% and a negative predictive value of 61% was obtained when using a cut-off value of 445 pmol/L. Clinically significant radiographic heart enlargement could be differentiated from those that did not, using a cut-off value of 680 pmol/L (positive predictive value, 81%; negative predictive value, 86%).

Commercial laboratory performing NT-proBNP assay maintains that heart disease is unlikely in patients with serum or plasma NT-proBNP is ≤566 pmol/L. Serum NT-proBNP >1200 pmol/L had a positive predictive value of 85.5% and a negative predictive value of 81.6% for distinguishing dogs with congestive heart failure from those with signs due to primary respiratory disease. In severe pulmonary disease and concurrent pulmonary hypertension, NT-proBNP can be falsely elevated and this has the potential to confound interpretation of the test results. Dogs with renal azotemia (and structurally normal hearts), had a serum mean NT-BNP level of 1069 pmol/L (range 179-2071), which was significantly elevated as compared to the normal group (mean 282 pmol/L, range 179-578 pmol/L). The degree of variation caused some dogs to occasionally test above the upper reference limit of 566 pmol/L. Thus, overtly healthy dogs, with only mild elevation of a single NT-proBNP test, may benefit from serial testing (Reynolds and Oyama, 2008).

In another study, NT-proBNP < 800 pmol/L was considered normal, 800-1800 had increased probability of heart disease, > 1800 had heart disease, and > 2700 had CHF. In people, BNP has greater sensitivity and specificity than X-ray and ECG and was more cost effective than echocardiography in detecting heart disease (Prosek and Ettinger, 2010). The primary end point occurred in 24% of patients in whom the BNP level was lowered, as compared with 52% of the control group, suggesting that therapy directed by BNP level is superior to guideline-directed therapy (Braunwald, 2008). Studies have shown that NT proBNP is an independent prognostic factor for the progression of heart failure in dogs (Ebisawa *et al*., 2012). Higher value at admission/ diagnosis carries poor prognosis (Serres *et al*., 2009).

Since several factors may influence BNP concentrations, sound clinical judgment and with the broad differential diagnosis in mind, both BNP and NT-proBNP have been shown to be of value for correctly identifying or excluding heart failure (Kimmenade and Januzzi, 2009).

2.3.2.2 Cardiac troponin-I (cTnI)

Cardiac troponin is one of the most valuable biochemical markers of myocardial damage (Linde *et al*., 2006).

The troponin complex is composed of 3 subunits (cTnI, cTnT, and cTnC) that help regulate excitation contraction coupling in the cardiac myocyte. Injury to the sarcomere causes detachment of cTnI from actin and subsequent disruption of the cellular membrane allows leakage of cTnI into the general circulation (Reynolds and Oyama, 2008). Its concentration in serum is correlated to the severity of myocardial damage (Oyama and Solter, 2004) and depends on release from myocardium, leakage into circulation and degradation by serum proteases, in kidney, liver and reticuloendothelial system (Prosek and Ettinger, 2010).

cTnI is detectable in blood 5-7 hours after injury, peaks at 1-2 days and dissipates by 1-2 weeks in humans with acute myocardial infarction (MI) and dogs with experimental MI (Adin *et al*., 2005). In another study, cTnI elevated in plasma within 4 hours of cardiac injury, peaked at 14-18 hours and persisted for 4-7 days. The half-life in dogs was less than 70 minutes (Linde *et al*., 2006).

Half life of troponin is 6 hours for I and 2 for T isoforms (Prosek and Ettinger, 2010).

CHF (without necrosis and lethal disruption of the sarcolemma) may cause ongoing degradation of myocardial troponin leading to progressive impairment of contractile function. cTnI is elevated earlier and more frequently than cTnT. As cTnI is elevated in many cardiac and non-cardiac diseases, they may be more useful in prognosis than diagnosis in the dog (Prosek and Ettinger, 2010).

The principal clinical use of concentrations of troponins in human patients is for the detection of myocardial ischemia secondary to coronary vascular disease, primarily atherosclerosis. This primary indication for the use of troponin is not a condition that occurs commonly in veterinary patients (Boswood, 2009). However they non-specifically identify evidence of myocardial damage rather than identifying a particular cause of the damage, limiting their utility as tests for discrimination between causes of myocardial injury. Despite the low incidence of ischemic heart disease in small animals as compared to people, cTnI appears useful for diagnosis of cardiac injury and mortality prognosis (Prosek and Ettinger, 2010).

Amino acid sequence of cardiac troponin is unique from skeletal muscle troponin and cTnI is a sensitive and specific biomarker for cardiac myocyte damage (Adin *et al*., 2005). It is exclusively present in cardiac muscle (Adin *et al*., 2005; Mellor *et al*., 2006). cTnI isoform of cardiac muscle is not expressed in skeletal muscle or other tissues and is 42-45% different from the skeletal isoforms (Prosek and Ettinger, 2010). It has greater

specificity for myocardial damage than previously used markers such as LDH and CK-MB (Reynolds and Oyama, 2008) and remains detectable for longer.

cTnI increases in arrhythmias, valvular disease, congenital subaortic stenosis, babesiosis, doxorubicin toxicity, gastric dilatation and volvulus, heat stroke, cardiomyopathy, myocarditis, blunt thoracic trauma (myocardial injury) and pericardial disease (Oyama and Solter, 2004; Adin *et al*., 2005; Mellor *et al*., 2006; Linklater *et al*., 2007). The mean cTnI in normal dogs has been 0.03 ng/mL in various studies (Oyama and Solter, 2004; Linde *et al*., 2006) and values up to 5.47 ng/dL have been recorded in MVD and DCM (Oyama and Solter, 2004) and up to 69.89 ng/mL in pericardial effusion (Linde *et al*., 2006).

Myocyte death, with release of cTnI whether through necrosis, apoptosis, or autophagy, is a feature of heart failure in both humans and animals, and is believed to play a key role in the development and progression of cardiac dysfunction (Linklater *et al*., 2007).

2.3.2.3 Creatine kinase – myocardial band (CK-MB)

The CK-MB enzyme consists of two subfractions, CK-MB1 and CK-MB2, known as CK-MB isoforms. CK-MB2 is believed to be the tissue-specific enzyme that is released into the serum when myocardial necrosis occurs and is subsequently converted to CKMB1. Baseline values in dogs were 10.7±6.6 IU/L (Elrifai *et al*., 1996).

Creatine kinase – myocardial band, an isoenzyme of creatine kinase (CK), is a marker of myocardial cell damage in humans and animals, however skeletal muscle injury can also lead to elevated CK-MB concentrations (Dolci and Panteghini, 2006). In normal

dogs, CK-MB reference range is 4.9- 6.3UI/L (Diniz *et al*., 2007). Normal values as given by Carreton *et al*., (2013) is < 17.5 IU/L. CK-MB is a less sensitive biomarker than cTnI for assessing myocardial damage (Tharwat *et al*., 2013).

2.3.3 Sample stability and interference

cTnI should be performed on same day unless frozen (Oyama and Solter, 2004). Oyama and Solter (2004) used heparinized plasma for measurement of troponin. Serum or heparinised plasma was used by Linde *et al*., (2006).

NT-proBNP was analysed from serum held frozen at -20 ^{o} C till analysis. For NT-BNP, EDTA plasma stored at -20 ^{o} C is suitable for up to 90 days (Reynolds *et al*., 2012). Heparinised plasma was used for cTnI and EDTA plasma for NT-proBNP (Trafny *et al*., 2012). A significant increase in NT-proBNP concentration after freezing would imply that reference ranges for normal canine NT-proBNP which have been derived mostly from serum that has been frozen at -20 ^{o} C or -70 ^{o} C may not be applicable when interpreting assay results of samples that have not been frozen (Collins *et al*., 2010).

2.3.4 Use of human assay kits

The amino acid sequence of canine troponin-I suggests that immunoassays designed for humans may be able to quantify canine cTnI (Oyama and Solter, 2004; Reynolds and Oyama, 2008). Some human assay kits have been validated in canines (Mellor *et al*., 2006). Increases in cTnI in canine heart diseases are typically milder than

those in myocardial infarction in man. The close homology of cTnI among mammals allows accurate measurement in dogs and cats using immunoassays developed for humans.

Canine pre-proBNP only shares 45% homology with human pre-proBNP. Hence human assay kits are not suitable for canines (Kimmenade and Januzzi, 2009).

2.3.5 Utility in asymptomatic patients

Although cTnI is elevated in many asymptomatic dogs, the lack of specificity of this test makes it unlikely to be useful as a standalone screening test (Boswood, 2008). Strategies that combine various biomarker assays, such as testing for both cTnI and NT-proBNP may provide a better diagnostic tool for asymptomatic patients (Reynolds and Oyama, 2008).

2.4 CONGESTIVE HEART FAILURE (CHF)

Ettinger and Suter (1970) defined congestive heart failure as the inability of heart muscle to maintain an adequate cardiac output- that is, to supply the needs of the body tissues.

The term congestive heart failure includes a spectrum of clinical manifestations resulting from circulatory congestion and edema due to heart disease, the cardinal feature of which is elevated filling pressure of one or both ventricles (Knight, 1994).

Two common causes of heart failure in dogs are mitral regurgitation associated with chronic acquired valvular disease and dilated cardiomyopathy (Ettinger *et al*., 1998).

Heart failure is a clinical syndrome in which impaired pumping decreases ventricular ejection and impedes venous return (de Morais, 2000).

The definition of heart failure according to the American Heart Association (AHA) guidelines for the evaluation and management of heart failure is “Heart failure is a complex clinical syndrome that can result from any structural or functional cardiac disorder that impairs the ability of the ventricle to fill with or eject blood” (DeFrancesco, 2002).

Heart failure or congestive heart failure is a general term that describes a clinical syndrome that can be caused by a variety of specific heart diseases (Atkins *et al*., 2009). Cardiac failure is the pathophysiologic state where in the heart is impaired in its ability to eject or receive blood, resulting in physical disability and the manifestation of a constellation of clinical signs that characterise the familiar clinical syndrome of heart failure. When abnormal cardiac function leads to the accumulation and retention of sodium and water with resulting congestion and edema, the term congestive heart failure is used (Sisson, 2010).

Heart failure can result from functional impairment of myocardium, the heart valves and the pericardium or as a consequence of increased resistance to ejection (Sisson, 2010). MVD accounts for approximately 75% of cases of CHF and the proportion is considerably higher in affected breeds (Olsen *et al*., 2010).

Congestive heart failure is a common and often fatal clinical syndrome in dogs characterized by cardiac dysfunction, neurohormonal activation, sodium and water retention, and increase in left ventricular filling pressures. It occurs most often secondary

to degenerative mitral valve disease (MVD) and dilated cardiomyopathy (DCM) (Schober *et al*., 2010).

Congestive heart failure is a condition that can result from any structural or functional cardiac disorder that impairs the ability of the heart to operate optimally. The most common causes of heart failure in dogs and cats are: valvular heart diseases, congenital heart diseases, primary cardiomyopathies, and heart worm infections (Hyun and Lavulo, 2011).

Neurohumoral factors such as catecholamines, the renin angiotensin-aldosterone system, endothelin and natriuretic peptides are involved in the pathophysiology of heart failure and in the maintenance of homeostasis (Kanno *et al*., 2012).

2.4.1 Classification of heart failure

Heart failure is classified into many categories based on severity and to guide therapy. These functional classification systems were designed to provide a framework for discussing and comparing the clinical signs of patients in heart failure. The systems may vary in their details, but they serve as semiquantitative schemes for judging the severity of a patient's clinical signs. Such categorization aids in teaching therapeutic protocols and constitutes a basis for stratification of subjects in clinical trials (Atkins *et al*., 2009).

2.4.1.1 New American Cardiac Council/ American Heart Association 2001 classification (Atkins *et al*., 2009)

Stage A: High risk for developing heart disease.

Stage B: Structural heart disease, no clinical signs

Stage B1: No radiographic or echocardiographic evidence of cardiac remodeling.

Stage B2: Radiographic or echocardiographic findings of left-sided heart enlargement.

Stage C: Clinical signs of heart failure associated with structural heart disease.

Stage D: End-stage disease with clinical signs that are refractory to ‘‘standard therapy’’

2.4.1.2 New York Heart Association guidelines (Atkins *et al*., 2009)

Class I: Asymptomatic heart disease.

Class II: Heart disease that causes clinical signs only during strenuous exercise.

Class III: Heart disease that causes clinical signs with routine daily activities or mild exercise.

Class IV: Heart disease that causes severe clinical signs even at rest.

2.4.1.3 Canine Heart failure International Expert Forum (CHIEF) system (Wolf *et al*., 2012)

Class A: Patients at risk for heart disease

Class B: Patients with structural heart disease, but no signs of CHF.

Class C: Past or current signs of CHF:

C1 have no current signs of CHF

C2 have mild to moderate CHF

C3 have severe to life-threatening CHF

Class D denotes patients with refractory heart failure.

2.4.2 Clinical signs of heart failure

Clinical signs in heart failure may result from accumulation of fluids, low cardiac output, or changes in skeletal muscles. Biventricular heart failure is often characterized by accumulation of pleural fluid (de Morais, 2000).

Cardiac cachexia is seen most commonly in dogs with DCM, especially those with right-sided CHF (Dukes-McEwan, 2000).

Cough is the most common clinical sign reported by the owner and usually occurs due to left atrial enlargement causing tracheal compression. The cough tends to be soft with cardiac disease, honking with tracheal disease and harsh and sometimes productive with bronchial disease (Ristic, 2004).

Clinical signs and physical examination findings of congestive heart failure are exercise intolerance, weakness, cyanosis, syncope, pale mucus membranes, weak femoral pulse, pulse deficit, respiratory crackles, cough, dyspnoea, tachypnoea, jugular venous distension, ascites, tachycardia, arrhythmia, systolic murmur, polydipsia, inappetance, weight loss, muscle wasting and elevated body temperature (Dukes-McEwan *et al*., 2003, Atkins *et al*., 2009). Heart rate increases significantly as heart disease worsens (Boswood and Murphy, 2006).

2.4.3 Laboratory findings

Hematology and biochemistry are not particularly useful for the diagnosis of heart disease (de Morais, 2000); however, they can be helpful to investigate potential concurrent disease.

Routine blood analysis may be within normal range. Prerenal azotemia may indicate low cardiac output. Circulating neurohormones are increased (Dukes-McEwan *et al*., 2003).

No significant changes in hematocrit are seen. Urea increases and sodium, potassium and chloride tend to decrease in more advanced heart failure (Boswood and Murphy, 2006).

Hematology and biochemistry panels are unremarkable in mild cases, but serious cases may have mildly elevated liver enzymes and prerenal azotemia (Olsen *et al*., 2010).

2.4.4 ECG findings in CHF

ECG is useful in the clinical evaluation of two major areas: the diagnosis of arrhythmias and conduction disturbances, and the status of the myocardium (Cohen, 1983; Tilley, 1992).

An ECG diagnosis of atrial fibrillation or left bundle branch block in the dog with clinical signs consistent with heart failure is suggestive of severe structural heart disease and heart failure. The finding of supraventricular or ventricular premature complexes or tachycardia is compatible with heart failure, but is not exclusive to a diagnosis of heart failure (DeFrancesco, 2002).

Boswood (2001) has reported that cardiac arrhythmias are commonly discovered in veterinary patients. These animals may be suffering from cardiac disease, non-cardiac disease or may be apparently normal.

The main indication for performing electrocardiography is to categorise arrhythmias noted during clinical examination (Ristic, 2004).

Atrial fibrillation is a common abnormality. Ventricular premature contractions and ventricular tachycardia are frequently reported in Boxers and Dobermans. Holter monitoring may be more useful, especially in the preclinical DCM cases (Dukes-McEwan *et al*., 2003).

Arrhythmias are more common in dogs with DCM since it is a disease of the myocardium. Atrial fibrillation is seen most commonly due to atrial enlargement. Certain breeds, such as Boxers and Dobermanns show ventricular arrhythmias (Ristic, 2004).

In a study by Oyama and Sisson (2004), 73% of dogs with cardiomyopathy had ventricular arrhythmias or atrial fibrillation.

Sinus tachycardia is a common feature (Boswood, 2008), particularly in the face of CHF (Meurs, 2010). Loss of sinus arrhythmia is common (Olsen *et al*., 2010).

Many dogs with heart disease may have normal recordings (Meurs, 2010; Olsen *et al*., 2010). ECG is an insensitive indicator of cardiac enlargement and cannot detect heart failure or pulmonary edema. ECG is limited in the diagnosis and management of MVD with the exception to document and classify an arrhythmia (Olsen *et al*., 2010). An abnormal heart may have a normal ECG and vice versa. ECG is not always an absolute indicator of normalcy or disease and patterns emerging from serial tracings or additional clinical information are essential for understanding the true meaning of most patients' ECGs (Cote, 2010).

Presence of atrial arrhythmias is indirect sign of elevated LA pressure (Chetboul and Tissier, 2012).

Cardiac arrhythmias are not common in dogs in early stages of MVD, although an increased frequency of supraventricular arrhythmias has been associated with increasing mitral valve prolapse in young Dachshunds and with left atrial enlargement in older dogs with advanced stages of MVD (Rasmussen *et al*., 2012).

2.4.5 Radiography in CHF

The abnormal cardiac silhouette produced in response to the physiological stress of abnormal circulation can be identified by thoracic radiography, even though it may be impossible to detect a specific cardiac problem (Hamlin, 1968).

Most radiographic assessments of the heart are performed with subjective analyses (eg, presence of a bulge, a vessel is larger than expected, increased sternal contact, or the heart shadow impinges upon the lung fields). Alternatively, efforts at semiquantitation are made (eg, the heart extends between the 3rd and 5th rib, or a pulmonary vein is larger than the corresponding pulmonary artery) (Nakayama *et al*., 2001).

Thoracic radiography is the single highest yield test in most coughing or dyspnoeic animals (DeFrancesco, 2002).

Thoracic radiography is the most commonly applied method for the diagnosis of CHF and is considered the clinical ‘‘gold standard’’ (Schober *et al*., 2010).

Left atrial (LA) enlargement, pulmonary venous congestion and pulmonary infiltrates compatible with cardiogenic edema are common signs (Linklater *et al*., 2007).

Pleural effusion and ascites are seen more commonly in DCM (Dukes-McEwan *et al*., 2003).

Cardiomegaly with enlarged left atrium, interstitial to alveolar pattern and dilated pulmonary veins consistent with cardiac pulmonary edema are seen in congestive heart failure (Wolf *et al*., 2012).

Increased left atrial dimension was assessed subjectively on lateral projection as dorsal elevation of the distal portion of the trachea and carina, dorsal displacement of the left bronchus, loss of caudal cardiac waist with the dilated chamber extending dorsally and caudally. On dorsoventral view, increased left atrial size was defined as increased opacity of the heart base caused by summation of the enlarged chamber and bulging of the left heart border at the 2–3 o'clock position (Ferasin *et al*., 2013).

Radiography is useful for the diagnosis of cardiopulmonary disease in small animals by providing reliable evidence of heart size and altered contours and pulmonary changes (Buchanan, 2013).

However, radiography is of unspecified sensitivity and specificity, especially in the setting of combined heart and lung disease, and can suffer from considerable observer variation (Schober *et al*., 2010; Ferasin *et al*., 2013).

2.4.6 Echocardiography

Echocardiography is useful in the evaluation of patients with congenital or acquired heart diseases and can be employed to estimate left ventricular function (Bonagura, 1983).

Echocardiography has the advantage over radiography in that it can look into the heart (Darke, 1992). Images are produced when the ultrasound beams are reflected from the fluid-soft tissue interfaces that a radiograph cannot discriminate.

DeFrancesco (2002) reported that the echocardiogram, though not essential for the diagnosis of heart failure, is a useful and non-invasive method for establishing the diagnosis of severe structural heart disease. It is a valuable technique for visualization of the cardiac anatomy (two dimensional echocardiography) and gaining both qualitative and quantitative insight into the systolic and diastolic cardiac functions (M-mode and spectral Doppler echocardiography) and blood flow (color and spectral Doppler). The echocardiogram is particularly useful in the diagnosis of pericardial effusion, dilated or hypertrophic cardiomyopathy, cardiac neoplasia and endocarditis.

The dogs are unsedated and placed in lateral recumbency with the cardiac area overlying a hole in the table to permit scanning from the dependent side. Right parasternal projections (long and short axis) are used to measure heart dimensions and evaluate valvular structures. The left atrial (LA) and aortic root (Ao) diameters during diastole are measured in a short-axis B-mode (2-D) projection. The measurements are obtained from the frozen image when the aortic and pulmonary valves are closed at diastole. The ratio between these two measures (La/Ao) is used as an index of atrial size. The left ventricular end diastolic and systolic diameters are measured in a short-axis M-mode projection of the heart in a plane just below the mitral valves (Haggstrom *et al*., 2000; Dukes-McEwan *et al*., 2003).

Conventional echocardiography is a non-invasive imaging technique that enables the investigation of cardiac morphology, hemodynamics, and contractile function.

Echocardiography is suitable for repeated measurements over time and thus enables easy non-invasive treatment follow-up (Chetboul *et al*., 2007).

Standard echocardiography is commonly performed on both humans and small animals to non-invasively assess myocardial function, and several bidimensional (2D) and M-mode measurements such as systolic left ventricular diameter and index volume or fractional shortening (%FS) are often used as indices of myocardial performance. Tissue Doppler imaging and its derived modalities, strain and strain rate imaging, are newly-developed ultrasound techniques permitting quantitative assessment of myocardial function. Two-dimensional speckle tracking echocardiography is an even more recent ultrasound modality based on 2D grayscale echocardiographic images. This non-invasive technique provides a new opportunity for the non-Doppler assessment of regional myocardial motion (Chetboul, 2008).

Echocardiography can identify and track many of the developments in heart disease and heart failure non-invasively and offers useful information about the heart and circulation (Bonagura and Schober, 2009).

Echocardiography in its various forms has become the most useful, non-invasive method for evaluating cardiovascular structure and function and diagnosing disease (Buchanan, 2013).

2.4.6.1 Echocardiographic criteria for diagnosis of DCM

Echocardiography is most sensitive method of confirming DCM. Diagnosis of DCM requires all of the following: (i) Left ventricular dilatation (ii) Reduced systolic function (iii) Increased sphericity of the left ventricle. Fractional shortening (FS) is used as

indicator of myocardial function in most studies. FS of < 20-25% is abnormally low. Ejection fraction (EF) of < 40% is also abnormal (Dukes-McEwan *et al*., 2003).

Criteria that are believed to be indicators of early disease include left ventricular size (left ventricular diastolic dimension > 4.6 cm, systolic dimension > 3.8 cm) in the Doberman (Meurs, 2003).

Left ventricular eccentric hypertrophy and loss of systolic function (Oyama and Solter, 2004) are characteristic of DCM.

The traditional measures of global LV systolic function are the ejection and shortening fractions. Ejection fraction (EF) is commonly measured by 2D echo using a single-plane (long axis or apical) image of the LV. Ejection fraction is calculated using end diastolic and end systolic volumes. These are calculated using single or biplane approach with the method of discs (Simpson's rule) to estimate LV volumes. The EF calculation informs us about the per cent of blood ejected from the LV during systole. Normal values for EF in healthy dogs using single-plane methods are approximately 45 to 55 per cent (Bonagura and Schober, 2009).

End diastolic frames correspond to onset of QRS, i.e. last frame before mitral valve closure and end-systolic frames correspond to end of T wave, i.e. last frame before mitral valve opening (Serres *et al*., 2009).

Shortening fraction (SF) or fractional shortening (FS) is measured in most situations from the M-mode echocardiogram but also can be determined from a frozen 2D image. The LV diameters are needed for calculation of SF. The SF is a linear estimate of EF, representing the percentage change of a single minor (internal) LV dimension from

diastole to systole. Normal values for SF are approximately 30 to 40 per cent for small breed dogs but probably as low as 22 to 25 per cent in larger breed dogs (Bonagura and Schober, 2009).

2.4.6.2 Echocardiographic criteria for diagnosis of MVD

Left ventricular eccentric hypertrophy and a regurgitant colour jet occupying the left atrium, thickened, nodular or prolapsing mitral valve leaflets, increased left atrial (LA) size and increased left atrium to aorta (LA/Ao) ratio are found during echocardiographic examination of the heart in CHF due to MVD (Oyama and Solter, 2004; Linklater *et al*., 2007; Chetboul and Tissier, 2012).

Abnormal protrusion of the mitral valve leaflets into the left atrium during systole, mitral valve prolapse, is usually identified by echocardiography at an early stage of the disease but worsens with progression (Madsen *et al*., 2011).

Mitral regurgitation (MR) severity is assessment by the maximal ratio of the regurgitant jet area signal to LA area (ARJ/LAA ratio) using color-flow Doppler mode. MR is usually considered as mild if the ARJ/LAA ratio is <20-30%, moderate if >20-30% but <70%, or severe if >70% (Chetboul and Tissier, 2012).

MR results in volume overload, characterised by LA enlargement and is measured as LA:Ao. LA:Ao is more than 1.6 in MVD (Reynolds *et al*., 2012).

Mitral valve lesions associated with MVD are firstly characterized by small, smooth nodules on the leaflet tips and thickened chordae tendineae with the nodules thickening and becoming more irregular during disease progression. MVD progression is

associated with increased sphericity of LV and decrease in LV sphericity index (Chetboul and Tissier, 2012).

Each of these systolic function indices (EF and FS) is unreliable in the setting of moderate to-severe MR due to MVD. In these situations, both EF and FS achieve values exceeding normal and the shortening area appears hyperdynamic when observed in real time (Bonagura and Schober, 2009).

Ejection fraction and fractional shortening are elevated. Normal values may indicate systolic myocardial failure. Presence of mitral valve thickening, prolapse, flail leaflet and mitral regurgitation are also seen (Trafny *et al*., 2012).

2.4.7 Therapy

The objectives of management of heart failure are to slow the progression of the disease, ameliorate existing signs and to reduce cardiac workload (Moser, 1989).

2.4.7.1 Conventional therapy

Kittleson (2000) stated that the primary aim of treating CHF is to reduce the formation of edema and effusions. In general, diuretics are the most efficacious of any drug class, and loop diuretics are the most efficacious diuretic type. The next most efficacious drugs are angiotensin-converting enzyme (ACE) inhibitors. A secondary goal in chronic heart failure patients is to increase cardiac output.

The main stay of management if CHF, irrespective of the cause is the combination therapy with furosemide and angiotensin-converting enzyme (ACE) inhibitors, with or without digoxin (Gordon *et al*., 2006; Oyama *et al*., 2009, Soares *et al*., 2010).

2.4.7.1.1 Diuretics

Diuretics are an integral component in the battle against CHF (Bright and Mears, 1997). Frusemide, a potent loop diuretic, has two methods of action. Initially, it causes venodilation which alters the vascular blood volume, shifting the blood from the pulmonary to the peripheral circulation. This shift decreases the pulmonary blood volume, pulmonary capillary pressure and pulmonary edema. Frusemide then diminishes the net absorption of fluid at the ascending loop of Henle in the nephron and promotes diuresis.

The cornerstone for treatment of CHF is furosemide (Haggstrom and Kvart, 2002). Frusemide is by far the most commonly used diuretic and the drug of choice for emergency management of pulmonary edema. Since excessive diuresis causes activation of RAAS, diuresis as a monotherapy is not recommended (Atkins, 2002).

Diuretics relieve signs of congestion, but they do little to alleviate and may exacerbate signs of forward failure (Boswood, 2008).

If frusemide is required at a dose exceeding 6 mg/kg per day, an additional diuretic should be used to act on a different part of the nephron. Potassium-sparing diuretics are useful in such a situation, although they are mild diuretics if used in isolation (Dukes McEwan, 2000). Resistance to diuretics is often overcome by addition of an aldosterone receptor antagonist like spironolactone (Haggstrom and Kvart, 2002). Specific aldosterone blocking agents such as spironolactone help mitigate the adverse effects of angiotensin II and aldosterone (Oyama, 2009). Improved survival and reduction of risk for a cardiac

event when under spironolactone treatment have recently been shown in dogs (de Madron *et al*., 2011).

Long-term treatment with spironolactone (2 mg/kg once a day) and conventional therapy (including at least an ACE inhibitor) is well tolerated in dogs with chronic HF. The addition of spironolactone to conventional therapy significantly reduced the number of deaths caused by cardiac disease, renal disease, or both and showed a trend toward reducing cardiorenal mortality (Lefebvre *et al*., 2013).

2.4.7.1.2 Angiotensin converting enzyme (ACE) inhibitors

ACE inhibitor enalapril extends the survival time or time to treatment failure (Ettinger *et al*., 1998). ACE inhibitors improve hemodynamics, clinical signs and exercise tolerance in heart failure (Pouchelon *et al*., 1999).

ACE inhibitors allow vasodilatation and reduce sodium and water retention by blocking the formation of angiotensin II and decreasing circulating aldosterone. The ACE inhibitors have the advantage over other vasodilators because they moderate excess neurohormonal responses (Ware and Keene, 1999).

Diuretics should never be used as monotherapy because they activate the RAAS. In addition, they should be used in conjunction with an ACE inhibitior (Dukes McEwan, 2000).

Studies indicate that ACE inhibitors are effective in combination with other therapy, and are now widely used for medical therapy of CHF in dogs (Kvart *et al*., 2002). ACE-inhibitors (enalapril, benazepril, ramipril) improve clinical scores of severity of heart

failure, such as modified NYHA class, indicating improved quality of life in DCM and MMVD dogs as adjunct therapy to other heart failure therapy. Furthermore, they have been shown to decrease treatment failure, i.e., either leading to death, euthanasia or worsening of signs of heart failure, in dogs with MR with ongoing heart failure therapy (Haggstrom and Kvart, 2002). They also improve the quality of life and extend life expectancy and delay the time to treatment failure (Amberger *et al*., 2004).

Traditionally, animals with CHF have been managed with judicious afterload-reducing agents such as ACE-inhibitors (Gordon *et al*., 2006).

Results of the veterinary enalapril trial prove reduction in onset of heart failure in dogs chronically treated with enalapril alone for compensated, naturally occurring mitral valve insufficiency (Atkins *et al*., 2007).

Efficacy and long-term tolerability of benazepril and other ACEI have been demonstrated convincingly in dogs by clinical trials involving large numbers of animals, especially those affected by degenerative mitral valve disease (MVD). Several prospective, double-blinded, multicentric, and randomized studies have shown that ACE inhibitors improve quality of life, increase exercise tolerance, and extend life expectancy in dogs with naturally acquired New York Heart Association class II-IV heart failure (Chetboul *et al*., 2007).

The renin angiotensin aldosterone system (RAAS) is generally stimulated in dogs with congestive heart failure secondary to DMVD. Either decreased renal blood flow or renal tubular sodium chloride concentration elicits production of preprorenin from the juxtaglomerular cells. Preprorenin is quickly cleaved to prorenin and then to renin by a trypsin-like enzyme. Renin converts angiotensinogen that is produced by the liver into

angiotensin I. Angiotensin I is then converted into angiotensin II (ATII) by angiotensin converting enzyme (ACE) as it passes through the pulmonary capillaries. The biological actions of ATII are contributory to the progression of heart disease and elevated ATII levels are predictive of cardiovascular death. By reducing activity of RAAS, ACE inhibitors improve outcome in dogs with symptomatic MVD (Oyama, 2009).

The classical therapy of DCM is based on diuretics, angiotensin-converting enzyme (ACE) inhibitors, and positive inotropes (digoxin) (Soares *et al*., 2010). The ACE inhibitors when combined with furosemide, significantly prolong survival and time to withdrawal from the study in dogs with CHF (de Madron *et al*., 2011).

2.4.7.1.3 Digoxin

Digitalis glycosides have been used in the dog since 1840s as a diuretic in the treatment of dropsy before its effect on the heart was clearly recognized (Ettinger and Suter, 1970). The digitalis glycosides are indicated for the treatment of myocardial failure and supraventricular arrhythmias.

The benefits of digoxin include a modest positive inotropic effect, supraventricular arrhythmia suppression and a direct sensitizing effect on atrial baroreceptors and reduction in sympathetic nerve traffic (Ware and Keene, 1999). Although it is intuitive that giving a positive inotrope to a patient with CHF should improve the cardiac output, it may often be deleterious and analogous to “flogging a dead horse” (Dukes McEwan, 2000).

2.4.7.2 Novel therapy

2.4.7.2.1 Nutritional therapy

Modulation of cytokine production is a potential means of managing cardiac cachexia. Dogs with CHF have been shown to have decreased concentrations of omega-3 fatty acids: eicosapentaenoic acid and docosahexaenoic acid. Long chain omega-3 fatty acids have important immunological, inflammatory and hemodynamic effects. In dogs with CHF, supplementation with omega-3 fatty acids decrease cytokine production, improve cachexia and may also prolong life (Roudebush and Freeman, 1999; Dukes McEwan, 2000; Ettinger, 2000; Elliott, 2002).

Taurine, L-carnitine, coenzyme Q10 and vitamin E (Roudebush and Freeman, 1999; Dukes McEwan, 2000; Ettinger, 2000; Dove, 2001; Elliott, 2002) have been used in the nutritional management of CHF.

Even though deficiency of carnitine or taurine are not documented in all cases of heart disease and failure, supplementation with the above nutraceuticals may help with energy metabolism in heart. In large or giant breed dogs, 2 grams of carnitine is administered orally with food q8-12 h. For American Cocker Spaniels (in combination with taurine), 1 g (1/2 tsp) carnitine q8-12h is sufficient. L-carnitine supplementation is generally continued for 3 to 6 months (Keene, 2002).

2.4.7.2.2 Pimobendan

Pimobendan is a positive inotrope, but also a vasodilator and hence is called a inodilator (Fuentes, 2004). This drug, unlike other positive inotropes, does not increase myocardial oxygen consumption. Pimobendan is an oral inotropic drug with

phosphodiesterase 3 inhibitory and calcium sensitizing effects. It causes increase in the extent of contraction for a given cytosolic concentration of calcium and a reduction in filling pressures and systemic vascular resistance (Fuentes *et al*., 2002).

Studies offer conflicting evidence with respect to the superiority of pimobendan for the treatment of CHF secondary to valvular disease (Gordon *et al*., 2006). Even though initial studies indicate that pimobendan appears to be useful in treating dogs with MR, long term studies involving larger number of dogs are needed to document improvement in quality of life (Kanno *et al*., 2007). Pimobendan is beneficial in dogs with left ventricular systolic failure, as demonstrated in Doberman Pinschers with dilated cardiomyopathy (Chetboul *et al*., 2007).

The therapeutic rationale behind the use of pimobendan in canine MVD relies on its preload and afterload reducing effect as well as the potential reduction of the mitral regurgitation (MR) through reduction of left ventricular (LV) size and enhancement of LV papillary and mitral annular tone (Caro *et al*., 2009; Ouellet *et al*., 2009).

Pimobendan causes a marked decrease in left ventricular (LV) end-diastolic pressure and pulmonary capillary wedge pressure in normal and heart failure dogs. Additionally, pimobendan causes potent vasodilatation of the systemic vasculature in dogs (Atkinson *et al*., 2009). Pimobendan therapy is well tolerated when added to standard congestive heart failure therapy.

Available prospective data overwhelmingly support its ability to significantly reduce morbidity in dogs with CHF secondary to DCM. The combination of drugs

including at least furosemide, an ACE inhibitor, and pimodendan has been recommended for the management of heart failure (de Madron *et al*., 2011; Summerfield *et al*., 2012).

2.5 PROGNOSIS

In human patients hospitalized for decompensated heart failure, the pre-discharge level of BNP was a strong, independent predictor of post-discharge outcomes. The primary end point occurred in 24% of patients in whom the BNP level was lowered, as compared with 52% of the control group, suggesting that therapy directed by BNP level is superior to guideline-directed therapy (Braunwald, 2008). Studies have shown that NT-proBNP is an independent prognostic factor for the progression of heart failure in dogs (Ebisawa *et al*., 2012) and higher value at admission/ diagnosis carries poor prognosis (Serres *et al*., 2009).

Prognostic indicators after the onset of congestive heart failure (CHF) are the required dose of furosemide, exercise intolerance, left atrium/aorta ratio (LA/Ao), serum creatinine concentration (Haggstrom *et al*., 2008; Serres *et al*., 2009). Clinical status at the first presentation for CHF was one of the best predictors of overall cardiac survival in dogs with MVD (Wolf *et al*., 2012). Systolic function is not a significant prognostic indicator (Dukes-McEwan *et al*., 2003).

MATERIALS AND METHODS

III. MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Patient selection

Dogs presented to Veterinary College Hospital, Bangalore from January 2014 to December 2014 either as primary cases or referred from other veterinarians were selected for the study.

3.1.2 Clinical Material

3.1.2.1 Blood

Blood was collected for hematology in clean and dry glass vials with ethylene diamine tetraacetic acid (EDTA) as the anticoagulant.

3.1.2.2 Serum

Blood for serum was collected in plastic blood collection tubes with clot accelerator (BD Vacutainer®). The sample was refrigerated till centrifugation and separation, which was done within 4 hours of collection and stored in aliquots for biochemical tests and biomarker analysis. Samples were frozen for cTnI and NT-pro BNP tests.

3.1.3 Laboratory material

3.1.3.1 Glassware

Glass vials of 5 mL capacity and test tubes were cleaned, washed and sterilized in hot air oven before use.

3.1.3.2 Anticoagulants

Di-sodium EDTA (S-d fine Chemicals, Boisar) was used as the anticoagulant at the rate of 1 mg/mL for collection of blood for hematology.

3.1.3.3 Reagents

Erba Mannheim® alanine aminotransferase (ALT), creatine kinase-myocardial band (CK-MB), creatinine, total protein, albumin, potassium and sodium kits were procured from Transasia Biomedicals Ltd, Himachal Pradesh.

Cardiac Troponin I (cTnI) from Lifescience Diagnostics, USA and amino terminal pro B-type natriuretic peptide (NT-proBNP) from Cusabio, China were sandwich ELISA kits that were procured for biomarker analysis. A rapid diagnostic kit based on immunochromatography for detection of human cTnI (i-tell®) was procured from Avinash Medicals, Bangalore.

3.1.3.4 Micropipette and pipette tips

Digital variable micropipettes and pipette microtips of 2-200 µL and 200-1000 µL capacity were obtained from Tarsons Products Pvt. Ltd., Kolkata.

3.1.3.5 Instruments

Fully automatic Blood Cell Counter PCE 210 (Erma Inc., Tokyo) was utilized for hematology and Trivitron Labmate 10 Plus semi-automatic biochemical analyzer (Trivitron Health Care, Bangalore) was utilized for biochemical analysis. ELISA reader (Biorad®) was utilized for reading the optical density (OD) of the ELISA plates.

3.1.4 Electrocardiographic equipment

MAC 400 (Wipro GE Healthcare Pvt. Ltd., Bangalore), 3 channel, 12 lead electrocardiograph was used to record the electrocardiograms. The electrocardiograph recording paper (Marquette Hellige Medical systems), a thermosensitive paper of a recording width of 75 mm was used. An electrically conductive gel (Medietech®, Kardia Cares, Chennai) was used as a conducting medium for application of electrodes. Commercially available crocodile clips used for connecting electrical circuits were modified and used to connect the electrodes of the electrocardiograph to the skin.

3.1.5 Echocardiography equipment

Logiq Book XP from General Electronics was used for echocardiography. A microconvex transducer of 6-10 Mhz capacity was used. A table with openings was used to scan the animal from the dependent side. A coupling gel (Medietech®, Kardia Cares, Chennai) was used to improve contact between the transducer and the animal and to improve image quality.

3.1.6 Radiography

Allengers 100 mA unit was used to obtain radiographs in lateral recumbency. Kodak X-ray films were utilized.

Other devices used were refrigerator, deep freezer and centrifuge for storage or processing of the samples.

3.2 METHODS

3.2.1 Selection of cases

Dogs presented with one or more signs of cardiac involvement like dyspnea, exercise intolerance, syncope, cyanosis, peripheral edema, ascites, cough and auscultable murmur were considered for the present study.

3.2.2 Collection of samples

Blood was collected from the cephalic or the saphenous vein using butterfly catheters and disposable syringes. Two millilitres of blood each was collected in EDTA and 2 mL for serum separation. Plasma was separated out from the EDTA sample for analysis of creatinine and ALT by centrifuging the sample at 2000 rpm for 5 minutes. Serum was separated 1-4 hours after collection by centrifugation at 2000 rpm for 5 minutes. Care was taken during collection and processing of blood samples to ensure that hemolysis did not occur. Serum samples were stored in aliquots at -20˚ C till further use.

3.2.3 Hematology

The total erythrocyte count and leukocyte counts, platelet count, hematocrit and hemoglobin were measured using fully automated Blood Cell Counter on the day of sample collection.

3.2.4 Blood biochemistry

EDTA plasma was used to determine ALT and creatinine on the day of blood collection. Trivitron Labmate 10 Plus semi-automatic biochemical analyzer and commercial reagent kits were used. Total protein, albumin, CK-MB, sodium and potassium were measured in serum from refrigerated or frozen samples.

cTnI and NT-proBNP were measured from serum samples that were preserved at -20º C till use. The protocol as per the manufacturer's instructions was followed (Appendix I and II).

Qualitative assessment of cTnI was made from serum samples by the use of the rapid diagnostic kit: i-tell®. Two drops of serum (approximately 50 µL) was added to the specimen well of the test device and the results read after 10 minutes. A pink line in both the test and control areas was considered positive for troponin (≥ 0.5 ng/mL of serum) where as a line in the control area only was considered negative. The test result was considered invalid if no line appeared in the control area.

3.2.5 Electrocardiography

The ECGs were recorded using the standard bipolar and augmented unipolar limb leads at 25 mm/s speed and interpreted as described by Tilley (1992). ECG was recorded with the animals in right lateral recumbency on a nonconductive surface with the leads being connected proximal to the olecranon on the caudal aspect of the appropriate foreleg and over the patellar ligament on the cranial aspect of the appropriate hind leg. The conducting gel was applied to the skin before connecting the electrodes. The electrodes were connected to the skin using modified crocodile clips. The sharp teeth of the clips were flattened and the jaws slightly bent apart to avoid pinching of the skin at the site of electrode application. This prevented muscle contraction and subsequent artifacts in the ECG. In animals with severe respiratory distress or orthopnoea, ECG was recorded in the sitting or standing position.

3.2.6 Radiography

The cases were subjected to radiographic examination based on physical examination and ECG findings. Survey radiographs were taken in the lateral views.

3.2.7 Echocardiography

A complete echocardiographic examination, (transthoracic 2D, M-mode, and color flow Doppler) was carried out using transducer of 6.0–10.0 MHz frequency as indicated by Borgarelli *et al*., 2008. Examinations were performed in conscious, unsedated dogs in right and left lateral recumbeny. The cardiac area was overlying the hole or opening in the table to permit scanning from the dependent side. In orthopnoeic dogs, the echocardiographic examination was postponed till the dog stabilised with the help of diuretic treatment.

M-mode recordings were obtained from short-axis views with the dogs positioned in right lateral recumbency. M-mode measurements were obtained according to the leading-edge-to-leading-edge method. The measurements included left ventricular internal diameter at diastole ($LVID_d$), left ventricular internal diameter at systole ($LVID_s$), fractional shortening (FS), left ventricular free wall thickness in diastole ($LVFW_d$), left ventricular free wall thickness in systole ($LVFW_s$), and E-point to septal separation (EPSS). Fractional shortening in per cent was calculated as ($LVID_d$-$LVID_s$)/ $LVID_d$ X 100.

The 2D echocardiograms were obtained in both short and long axis views from the right and the left sides. The EDV and ESV were calculated by the modified Simpson's rule method. The LA/Ao was obtained from the 2D short-axis view. Long-axis images which optimised LV length and area was recorded from the right parasternal long axis view. A diastolic frame was selected and the endocardial border traced, closing across the mitral annulus. The LV length and volume (EDV) was thus measured at end diastole. The same procedure was followed for the subsequent systolic frame (smallest LV chamber). This gave the end-systolic volume (ESV). The ejection fraction was calculated as: (EDV-ESV)/EDV x 100% (Dukes McEwan *et al*., 2003; Serres *et al*., 2009). Mitral regurgitation was graded as mild, moderate or severe based on colour Doppler studies.

3.2.8 Treatment trials

Out of the dogs that were confirmed to be suffering from heart failure by a combination of physical examination and tests (electrocardiography, radiography and echocardiography), 24 dogs were randomly selected and subjected to treatment trials by allotting them to one of the 4 treatment groups as follows:

Group I: Conventional (diuretics frusemide and spironolactone, angiotensin converting enzyme (ACE) inhibitor enalapril with or without digoxin)

Group II: Conventional + Pimobendan

Group III: Conventional + Nutraceutical (Cardiostrength®)

Group IV: Conventional + Pimobendan + Nutraceutical (Cardiostrength®)

The doses of the drugs used were: frusemide (Lasix® 40 mg tabs, Hoechst Marion Roussel): 2-4 mg/kg BW twice or thrice a day, frusemide with spironolactone (Lasilactone® 50 containing 50 mg of spironolactone and frusemide 20 mg, Hoechst Marion Roussel): 1-2 mg/kg BW twice or thrice a day, enalapril (Envas® 2.5, 5, 10, 20 mg tabs, Cadila Pharma): 0.5 mg/ kg BW twice a day, pimobendan (Vetmedin ®, Boehringer Ingelheim and Safeheart®, Sava Vet 5 mg tab) 0.25 mg/kg BW twice a day, digoxin (Lanoxin® 0.25 mg tabs, Burroughs Wellcome): 0.005 to 0.01 mg/kg BW twice a day and Cardiostrength® (Vetri-science Laboratories, USA) 1 cap/ 10 kg BW once a day.

The active ingredients of Cardiostrength® were: L-carnitine HCl, 125 mg, L-taurine 125 mg, N,N-dimethylglycine HCl 25 mg, d-alpha tocopheryl succinate (Vitamin E) 30 IU, coenzyme Q10 10 mg, folic acid 0.9 mg, magnesium citrate 0.5 mg, potassium citrate 0.1 mg and sodium selenite 0.007 mg per capsule.

The cases were followed up once a month for 2 months. To evaluate the efficacy of therapy, 6 clinical variables were selected viz. ascites, anorexia, coughing, dyspnea, survival and activity. For each variable other than survival, a score of 0 was given if there was complete resolution of clinical signs, 1 if there was improvement but without complete resolution, 2 if there was no improvement and 3 if the condition worsened during therapy. For survival, a score of 0 was given if the dog was alive at the end of study period and 3 if it died during the study period. The mean of the scores for each variable was used to grade response to therapy and for comparison between groups.

3.2.9 Statistical analysis

The data obtained were analysed using “t-test” and chi-square test using GraphPad Prism software.

RESULTS

IV RESULTS

The results of this study are as detailed below:

4.1 Occurrence of congestive heart failure

A total of 17342 dog cases were presented to the Veterinary College Hospital in the year 2014 (January 2014 to December 2014). These were primary cases that were presented directly for the first time to the hospital and also referral cases that were initially examined by other veterinarians. Out of these, 95 dogs were suspected to suffer from congestive heart failure based on their history and clinical signs. Definitive diagnosis made based on physical examination, hematology, blood biochemistry, electrocardiography, radiography and echocardiography revealed that congestive heart failure occurred in 78 of these cases. The rest were diagnosed as cases of hepatic disease/ failure (7), renal failure (3), anemia (2), pulmonary neoplasm (2), pyometra with renal failure (1), pericardial effusion (1) and pneumothorax (1). The same is depicted in Table 1 and Fig. 1. In the current study, the occurrence of congestive heart failure was 0.45% out of the total number of cases presented to the Hospital. The occurrence out of suspected cases was 82.11 %. Out of 78 cases of congestive heart failure, 59 (76 %) were diagnosed as DCM and 19 (24%) as MVD. The incidence of MVD was 0.11 % and DCM 0.34 % out of the total cases for the year 2014. The same is presented in Table 2 and Fig. 2

4.1.1 Breed-wise occurrence of congestive heart failure due to DCM and MVD

The breed-wise occurrence of congestive heart failure in dogs is presented in Table 3 and Fig. 3. The occurrence was highest in the Labrador Retriever (25, 32.05%), followed by Non-descript dogs (17, 21.80%), Pomeranian (8, 10.25%),

Table 1: Occurrence of CHF among diseases presented with similar clinical signs (n=95)

Disease	No. of cases	Per cent
CHF	78	82.11
Hepatic disease	7	7.37
Renal failure	3	3.15
Anemia	2	2.11
Pulmonary neoplasm	2	2.11
Pyometra with renal failure	1	1.05
Pericardial effusion	1	1.05
Pneumothorax	1	1.05
Total	95	100

Fig. 1: Occurrence of CHF among diseases presented with similar clinical signs (n=95)

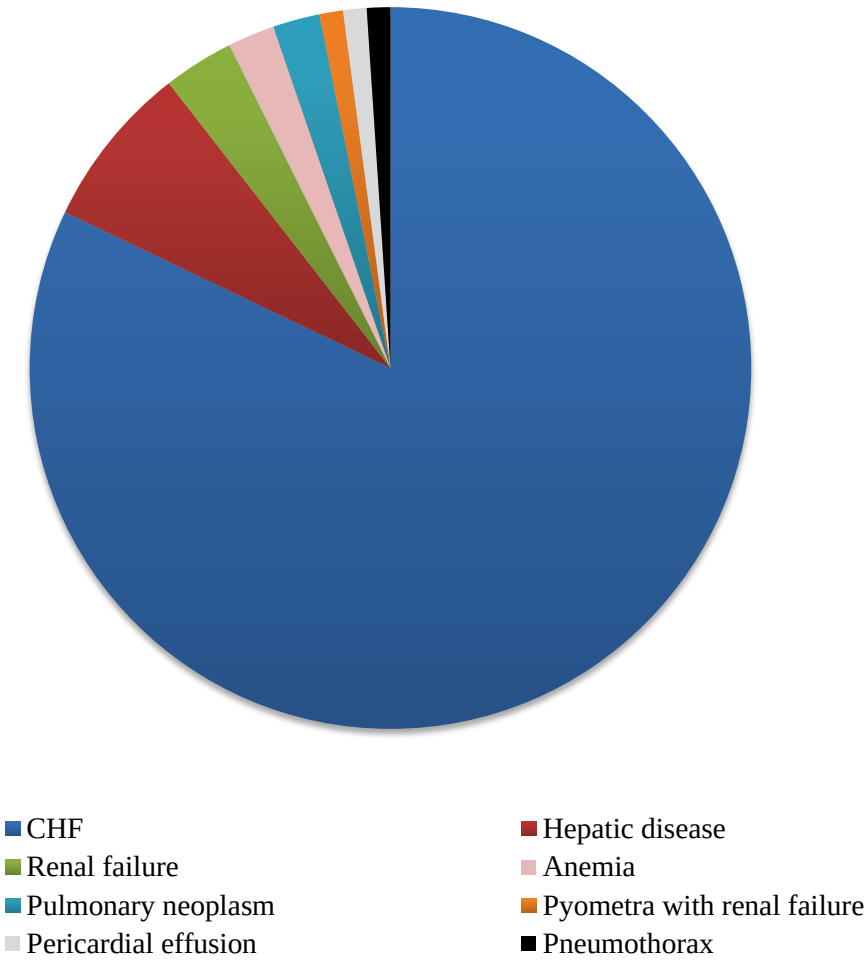
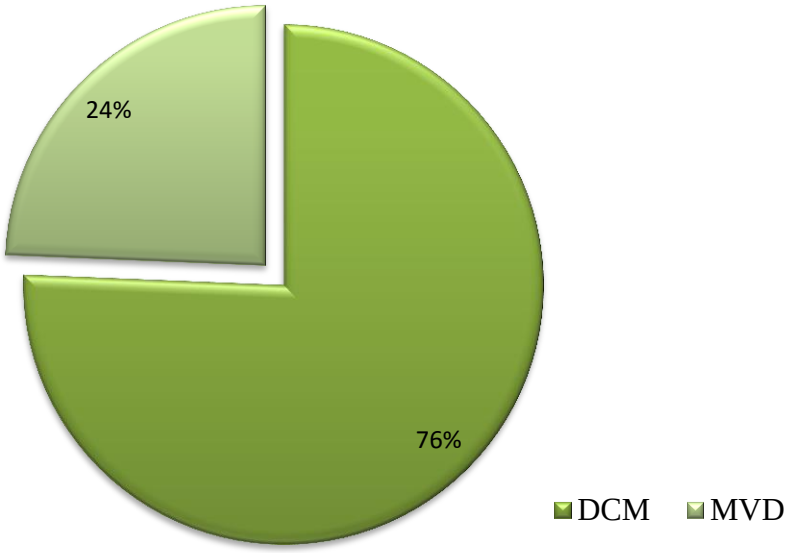


Table 2: Occurrence of CHF due to DCM and MVD in dogs (n=78)

Cause	No. of dogs with CHF	Per cent of CHF dogs	Per cent occurrence in 2014
DCM	59	76	0.34
MVD	19	24	0.11
Total	78	100	0.45

Fig. 2: Occurrence of CHF due to DCM and MVD in dogs (n=78)



Golden Retriever (7, 8.98%), German Shepherd (4, 5.12%), Mastiff, Doberman Pincher, Saint Bernard, Dachshund (3 each, 3.85%), Boxer (2, 2.56%) and Cocker Spaniel, Toy Poodle and Rottweiler (1 each, 1.28 %).

The breed-wise occurrence of MVD was Pomeranian (8), Non-descript dog (6), Dachshund (3), Cocker Spaniel (1) and Toy Poodle (1). The per cent occurrence among MVD and CHF is depicted in Table 3.

The breed-wise occurrence of DCM was Labrador Retriever (25), Non-descript dog (11), Golden Retriever (7), German Shepherd (4), Mastiff, Doberman Pincher, Saint Bernard (3 each), Boxer (2) and Rottweiler (1). The per cent occurrence among MVD and CHF is depicted in Table 3.

4.1.2 Age-wise occurrence of congestive heart failure due to DCM and MVD

The age-wise occurrence of congestive heart failure in dogs is presented in Table 4 and Fig. 4. The occurrence was highest in the dogs of 5-10 years of age (42, 54 %) followed by < 5 years of age (23, 29 %) and more than 10 years of age (13, 17 %).

The age-wise occurrence among MVD dogs was 0% in less than 5 year olds, 53 % (10) in 5-10 year old dogs and 47 % (9) in more than 10 year olds. The age of these dogs ranged from 7 to 14 years with the mean age of occurrence being 10.39 ± 0.51 years.

The age-wise occurrence among DCM dogs was 39 % (23) in less than 5 year olds, 54 % (32) in 5 to 10 year olds and 7 % (4) in more than 10 year olds. The age of these dogs ranged from 1.5 to 12.0 years and the mean age of occurrence being 6.26 ± 0.31 years.

The mean age of occurrence was significantly different between MVD and DCM dogs ($P < 0.05$).

Table 3: Breed-wise occurrence of CHF due to DCM and MVD in dogs (n=78)

Breed	**DCM**	**MVD**	**CHF**
Labrador Retriever	25 (42.37)	0 (0)	25 (32.05)
Non-descript dog	11 (18.64)	6 (31.57)	17 (21.8)
Pomeranian	0 (0)	8 (42.12)	8 (10.25)
Golden Retriever	7 (11.87)	0 (0)	7 (8.98)
German Shepherd	4 (6.78)	0 (0)	4 (5.12)
Mastiff	3(5.08)	0 (0)	3 (3.85)
Doberman Pincher	3 (5.08)	0 (0)	3 (3.85)
Saint Bernard	3 (5.08)	0 (0)	3 (3.85)
Dachshund	0 (0)	3 (15.79)	3 (3.85)
Boxer	2 (3.4)	0 (0)	2 (2.56)
Cocker Spaniel	0 (0)	1 (5.26)	1 (1.28)
Toy Poodle	0 (0)	1 (5.26)	1 (1.28)
Rottweiler	1 (1.7)	0 (0)	1 (1.28)
Total	59 (100)	19 (100)	78 (100)

Note: Figures in parentheses indicate percentage

Fig. 3: Breed-wise occurrence of CHF due to DCM and MVD in dogs (n=78)

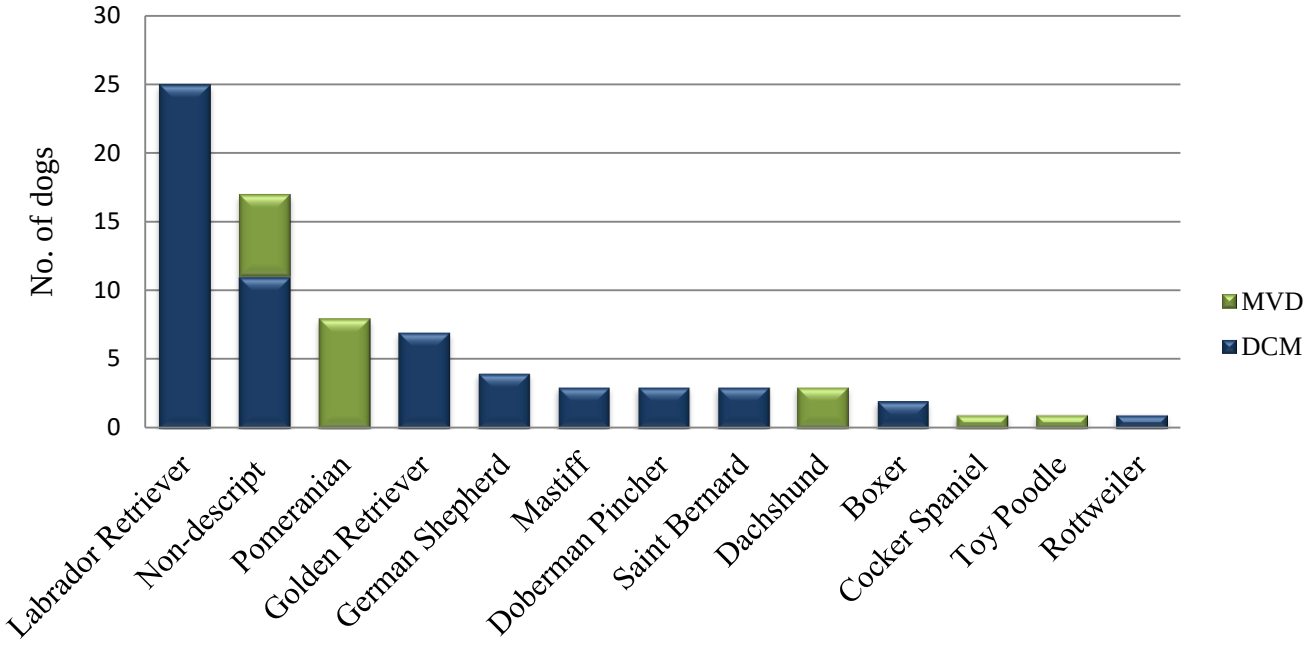
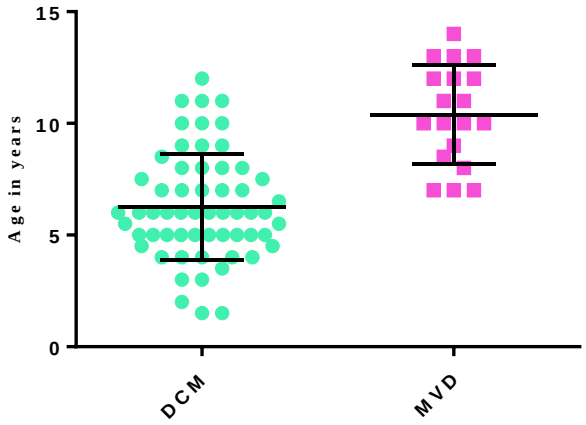


Table 4: Age-wise occurrence of CHF due to DCM and MVD in dogs (n=78)

Age	No. of CHF dogs	DCM	MVD
< 5 years	23 (29)	23(39)	0 (0)
5-10 years	42 (54)	32 (54)	10 (53)
>10 years	13 (17)	4 (7)	9 (47)
Total	78 (100)	59 (100)	19 (100)

Note: Figures in parentheses indicate percentage

Fig. 4. A scatter plot depicting age-wise occurrence of CHF due to DCM and MVD in dogs (n=78)



4.1.3 Gender-wise occurrence of congestive heart failure due to DCM and MVD

The gender-wise occurrence of cardiac disease in dogs is presented in Table 5 and Fig. 5. The occurrence in male dogs was 69.23 % (54 dogs) and 30.77 % in female dogs (24 dogs). Among MVD dogs, 13 (68.42 %) were male and 6 (31.58 %) were female. Among DCM dogs, 41 (69.49%) were male and 18 (30.51%) were female. There was a significant difference in the occurrence of CHF between males and females ($P < 0.05$).

4.2 Clinical signs of congestive heart failure in dogs

The clinical signs of congestive heart failure in dogs are presented in Table 6 and Fig. 6. Coughing was the most common clinical sign (32 dogs, 23.4%), followed by ascites (25 dogs, 18.3%), anorexia and dyspnoea (18 dogs each, 13.2%), exercise intolerance (8 dogs, 5.9%), epistaxis/ hemoptysis (7 dogs, 5.1%), tachypnoea (6 dogs, 4.4%), peripheral edema and lethargy (4 dogs each, 3.0 %), wheezing/ noisy respiration, orthopnoea and syncope (3 dogs each, 2.1%), pallor and cyanosis (2 dogs each, 1.4%) and weight loss and diarrhoea (1 dog each, 0.7%).

Table 7 and Fig. 7 depict auscultable abnormalities in cases of CHF. Auscultable abnormalities of heart included tachycardia (35 dogs, 44.87%), murmurs (16 dogs, 20.51%), gallop rhythm (5 dogs, 6.41%) and muffled heart sounds (5 dogs, 6.41%). Crackles due to pulmonary edema was seen in 7 (8.97%) dogs.

4.3 Classification of dogs with congestive heart failure

The dogs with congestive heart failure were classified as per the Canine Heart failure International Expert Forum (CHIEF) system given by Wolf *et al*., in 2012. Dogs with mild to moderate signs were classified as “C2” and in the present study, 63 dogs (80.77%) belonged to this group of which MVD was seen in 18 cases and DCM in 45.

Table 5: Gender-wise occurrence of CHF due to DCM and MVD in dogs (n=78)

Gender	**DCM**	**MVD**	**Total**
Male	41 (69.49)	13 (68.42)	54 (69.23)
Female	18 (30.51)	6 (31.58)	24 (30.77)
Total	59 (100)	19 (100)	78 (100)

Note: Figures in parentheses indicate percentage

Fig. 5: Gender-wise occurrence of CHF due to DCM and MVD in dogs (n=78)

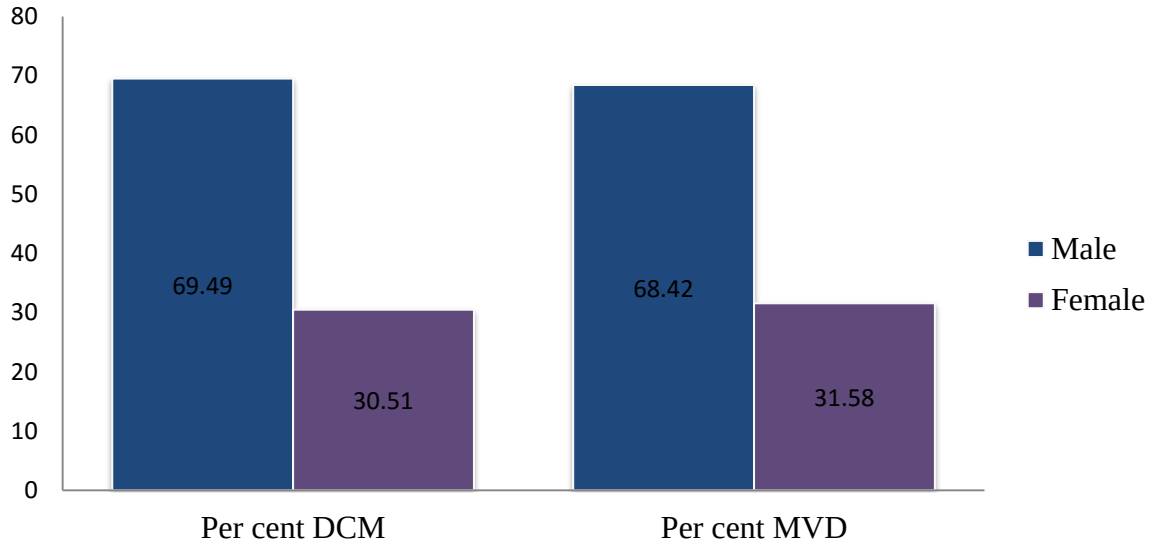


Table 6. Clinical signs manifested in CHF dogs (n=78)

Clinical signs	No. of dogs	Per cent
Coughing	32	23.4
Ascites	25	18.3
Anorexia	18	13.2
Dyspnoea	18	13.2
Exercise intolerance	8	5.9
Epistaxis/ hemoptysis	7	5.1
Tachypnoea	6	4.4
Peripheral edema and	4	3
Lethargy	4	3
Wheezing/ noisy respiration	3	2.1
Orthopnoea	3	2.1
Syncope	3	2.1
Pallor	2	1.4
Cyanosis	2	1.4
Weight loss	1	0.7
Diarrhoea	1	0.7

Fig. 6. Clinical signs manifested in CHF dogs (n=78)

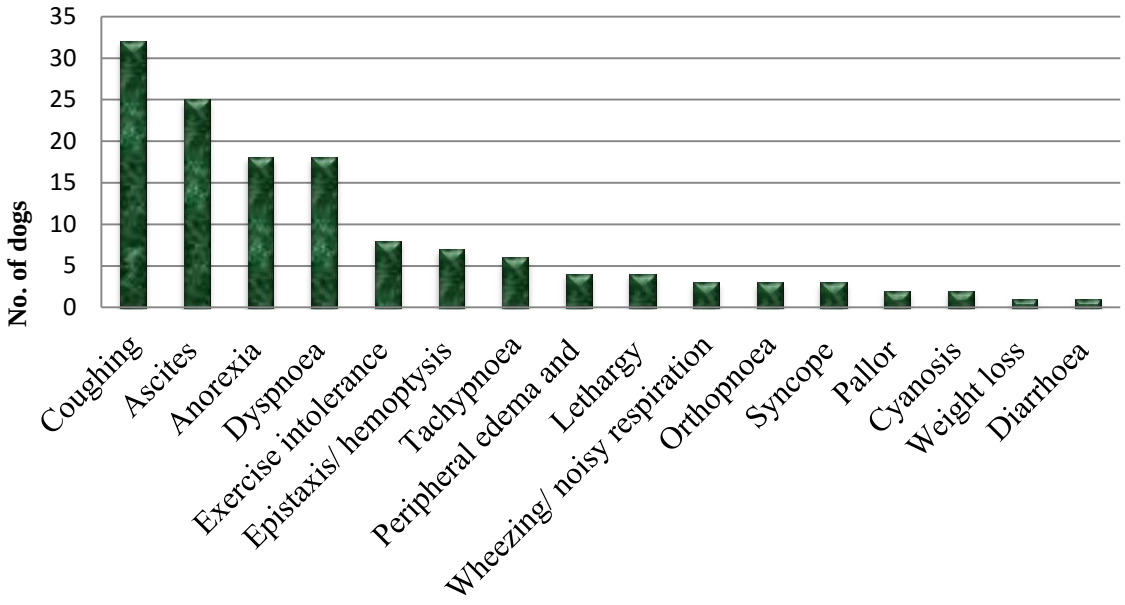
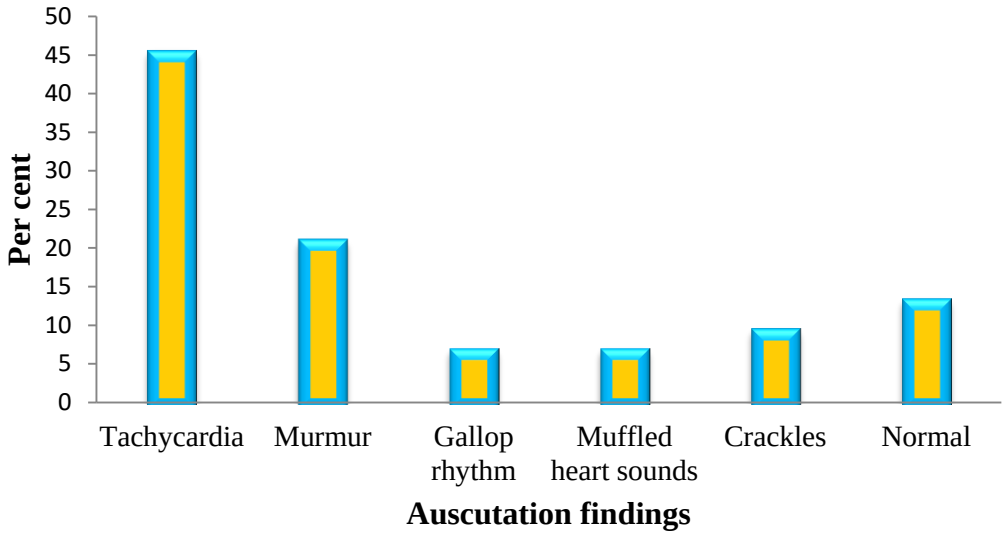


Table 7: Rhythm and sounds heard on auscultation of thorax in CHF dogs (n=78)

Auscultation findings	**No. of dogs**	**Per cent**
Tachycardia	35	44.87
Murmur	16	20.51
Gallop rhythm	5	6.41
Muffled heart sounds	5	6.41
Crackles	7	8.97
Normal	10	12.83
Total	78	100

Fig.7: Rhythm and sounds heard on auscultation of thorax in CHF dogs (n=78)



Dogs with severe to life-threatening HF were classified as “C3” and 13 dogs (16.67%) belonged to this group of which 1 had MVD and the rest 12 DCM. Two dogs (2.56%) with refractory heart failure were classified as “D” and these had DCM. This classification has been presented in Table 8 and Fig. 8.

4.4 Electrocardiographic findings in dogs with congestive heart failure

The electrocardiographic findings in dogs with CHF due to MVD and DCM have been depicted in Table 9, 10 and Figs. 9 to 22. Arrhythmia was seen in 49 cases and sinus tachycardia was the most common abnormality (29 dogs, 59.18 %), followed by atrial fibrillation (14 dogs, 28.57 %), ventricular premature complexes (2 dogs, 4.09%), atrial flutter (1 dog, 2.04%), ventricular tachycardia (1 dog, 2.04%), right bundle branch block (1 dog, 2.04 %) and premature atrial contraction (1 dog, 2.04%). Morphological abnormalities were seen in 17 cases and included tall R waves, i.e. more than 2.5-3.0 mV depending on the breed (7 dogs, 41.2%), tall T waves, i.e. more than one fourth the R wave (5 dogs, 29.1%), short R waves, i.e. less than 0.5 mV (2 dogs, 11.7%), and one each of deep Q, deep S (i.e. more than 0.5 mV) and ST segment sagging (6%). Two dogs which had sinus tachycardia at the first visit developed atrial fibrillation by the second visit.

4.5 Radiographic findings in dogs with congestive heart failure

The radiographic findings in 59 (47 DCM and 12 MVD dogs) dogs subjected to thoracic radiographs are depicted in Table 11 and Fig. 23-28. The findings included cardiomegaly in 51 dogs (39 DCM and 12 MVD dogs, 86.44%), elevated trachea (in 39 dogs (30 DCM and 9 MVD dogs, 66.1%), pulmonary edema/ congestion (hypervascularisation of lungs) in 42 dogs (31 DCM and 11 MVD dogs, 71.18%), reduced cardiophrenic angle in 39 dogs (36 DCM and 3 MVD dogs, 66.1%) and pleural effusion

Table 8: Classification of CHF dogs by CHIEF system (n=78)

Class	No. of dogs		Per cent
	DCM	MVD	
A	0	0	0
B	0	0	0
C1	0	0	0
C2	45	18	80.77
C3	12	1	16.67
D	2	0	2.56
Total	59	19	100

Fig. 8: Classification of CHF dogs by CHIEF system (n=78)

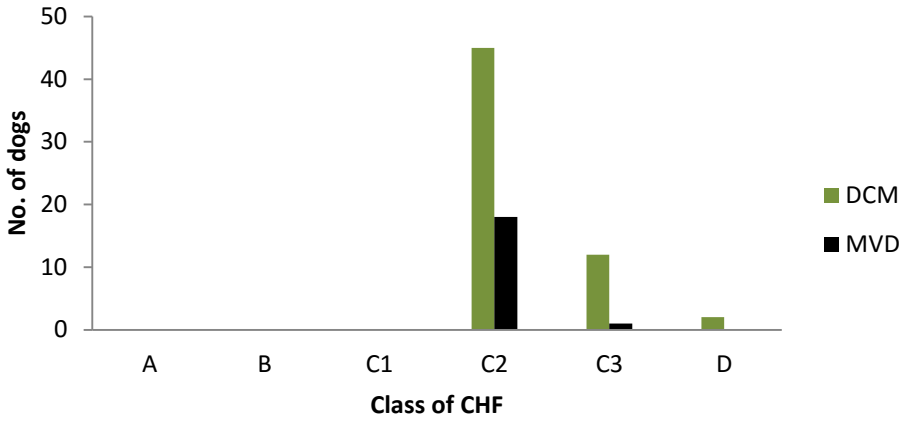


Table 9: Arrhythmias in CHF dogs (n=49)

Abnormal rhythm	**No. of dogs**	**% of abnormal rhythm**
Sinus tachycardia	29	59.18
Atrial fibrillation	14	28.57
Ventricular premature complexes	2	4.09
Atrial flutter	1	2.04
Ventricular tachycardia	1	2.04
Right bundle branch block	1	2.04
Premature atrial contraction	1	2.04
Total	49	100

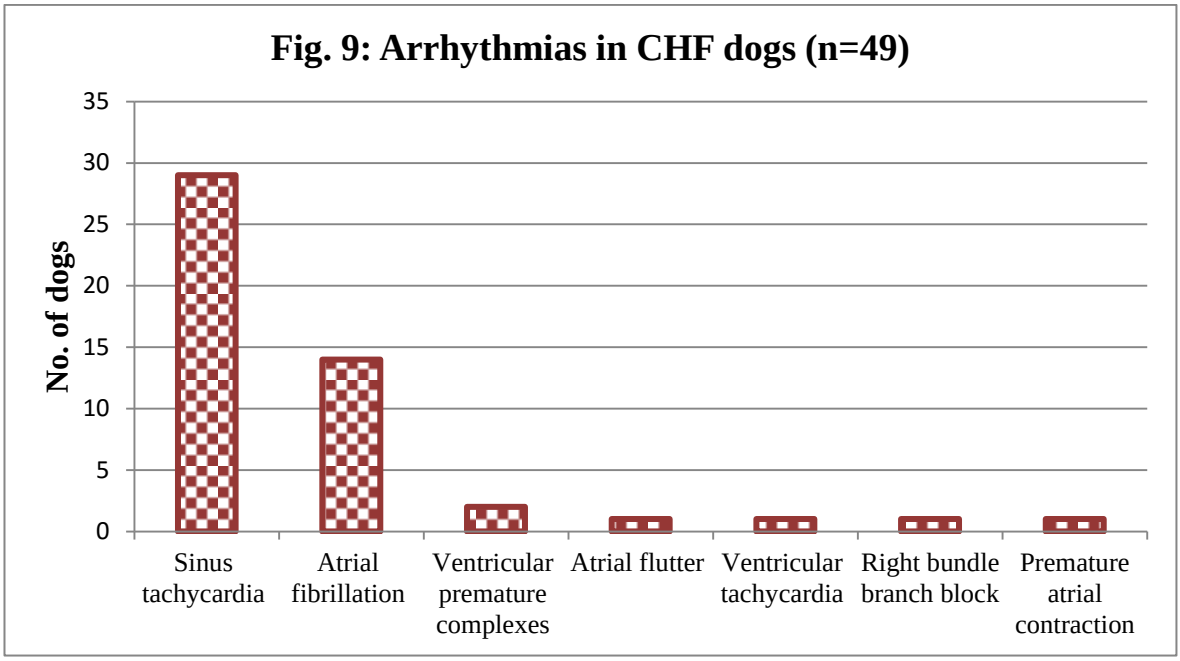
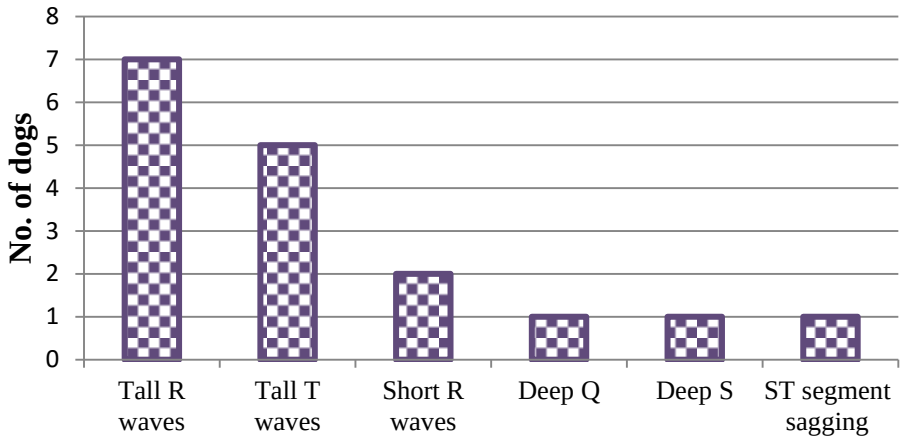


Fig. 9: Arrhythmias in CHF dogs (n=49)
No. of dogs
35
30
25
20
15
10
5
0
Sinus tachycardia
Atrial fibrillation
Ventricular premature complexes
Atrial flutter
Ventricular tachycardia
Right bundle branch block
Premature atrial contraction

Table 10: ECG abnormal morphology in CHF dogs (n=17)

Abnormal morphology	**No. of dogs**	**% of abnormal morphology**
Tall R waves	7	41.2
Tall T waves	5	29.1
Short R waves	2	11.7
Deep Q	1	6
Deep S	1	6
ST segment sagging	1	6
Total	17	100

Fig. 10: ECG abnormal morphology in CHF dogs (n=17)



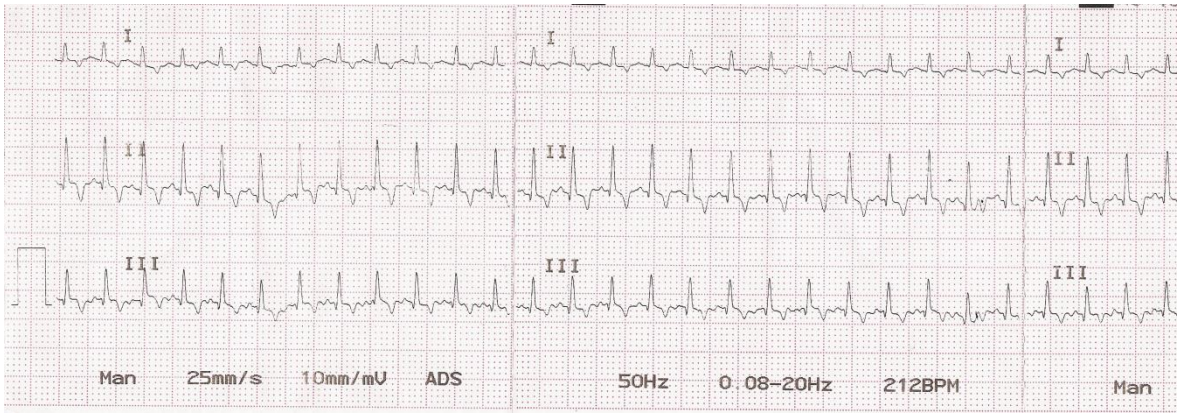


Fig. 11: ECG: Sinus tachycardia with heart rate 212 bpm (paper speed 25 mm/s, 1 cm=1mV)

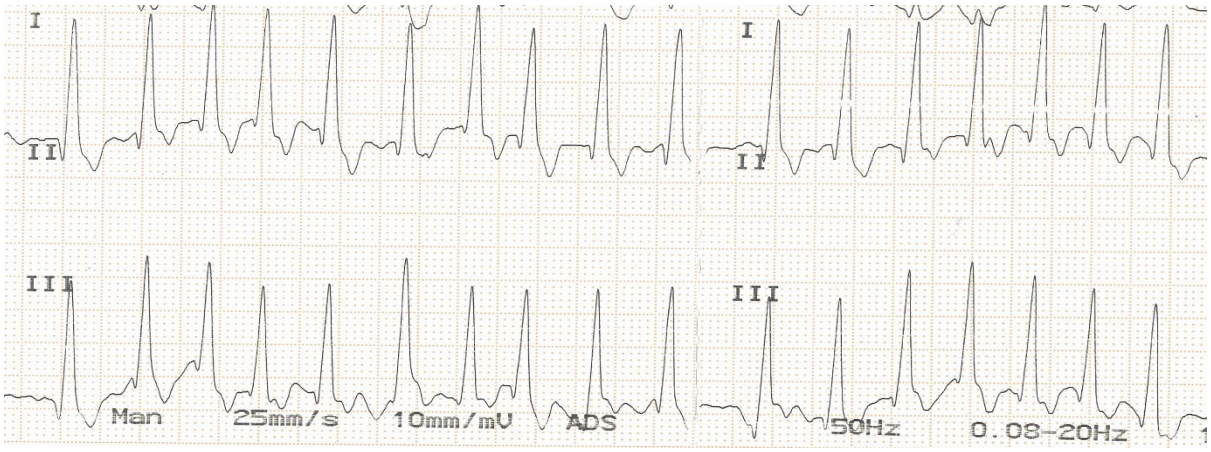


Fig. 12: ECG: Atrial fibrillation with ventricular rate 170 bpm (paper speed 25 mm/s, 1 cm=1mV)

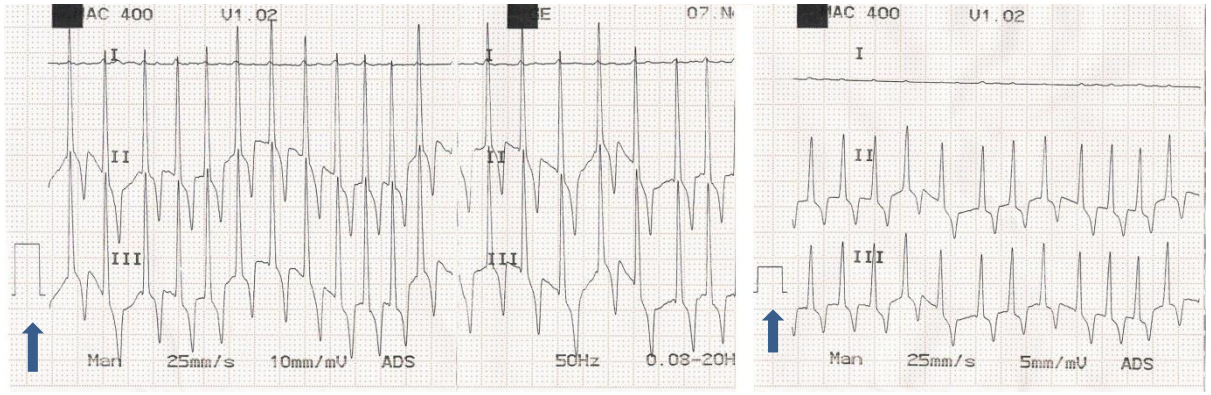


Fig. 13: ECG: Atrial fibrillation and tall R waves. Note: the arrows indicate that the second half of the recording is done at half the amplitude, R= 2.6 mV

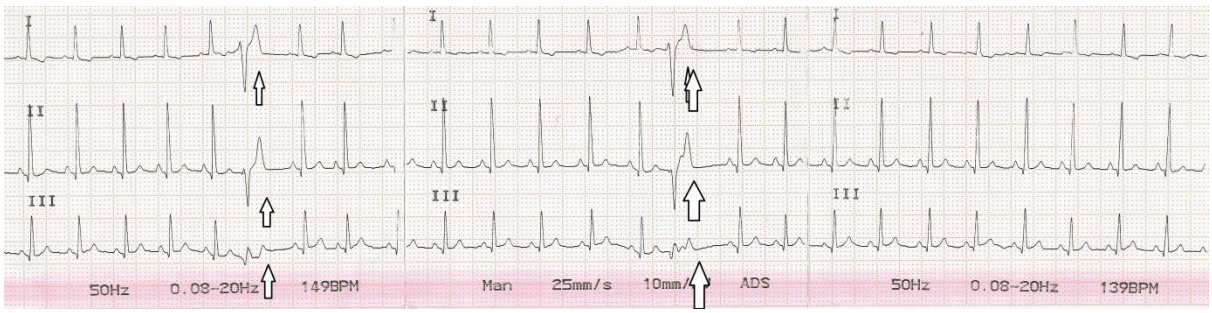


Fig.14: ECG: Ventricular premature complexes indicated by arrows showing large bizarre complexes occurring without a P wave preceding them

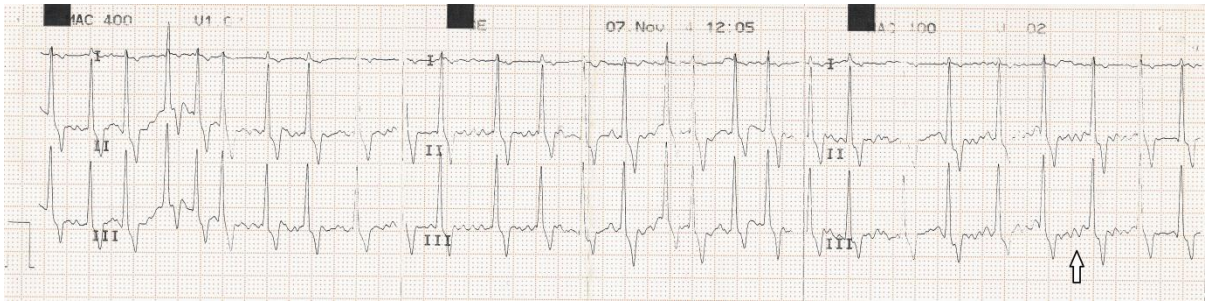


Fig.15: ECG: Atrial flutter characterised by small "F" waves indicated by arrow

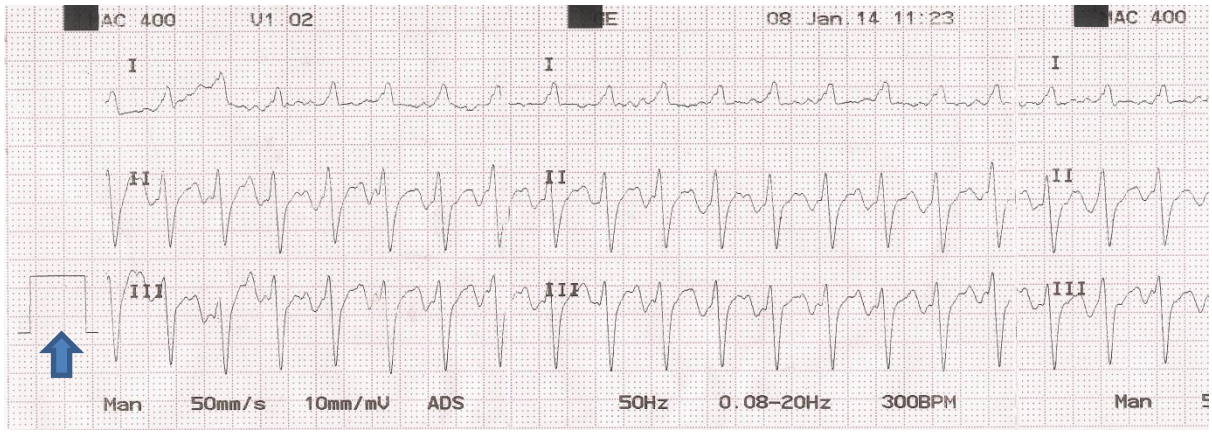


Fig.16: ECG: Ventricular tachycardia with 300 bpm ventricular rate. Note arrow indicating paper speed of 50 mm/s

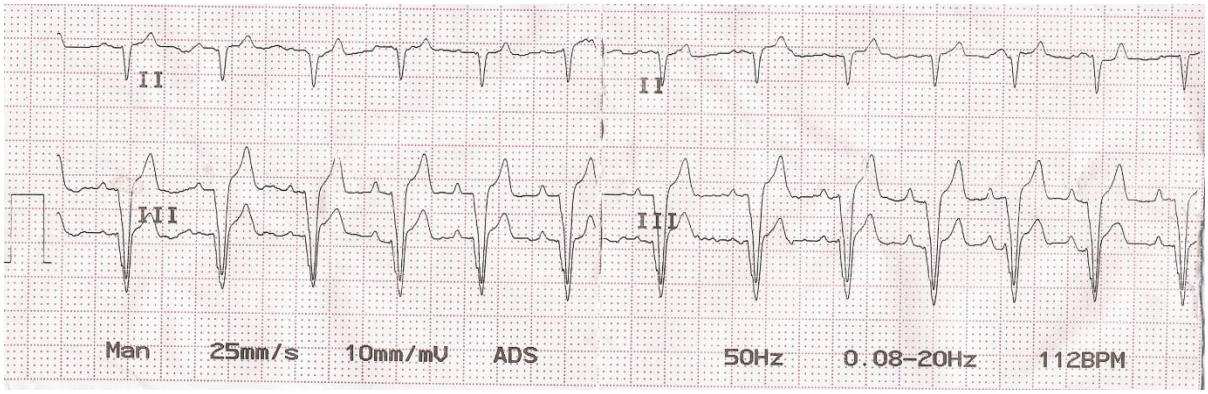


Fig. 17: ECG: Right bundle branch block. Note P waves preceding every bizarre QRS complex (Heart rate is 112 bpm)

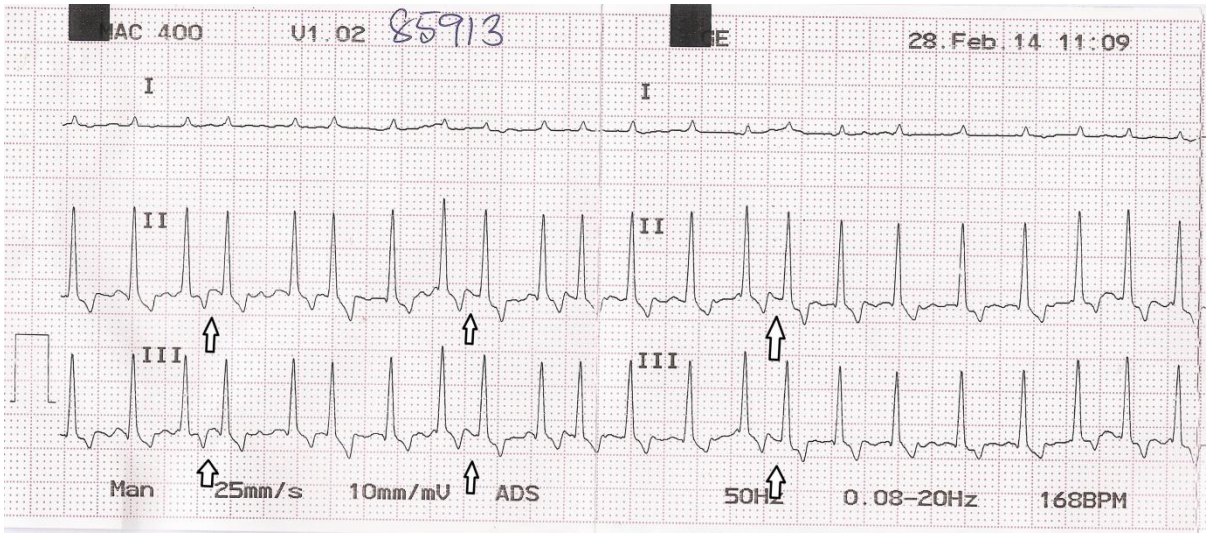


Fig. 18: ECG: Premature atrial contractions characterised by "P on T" indicated by arrows

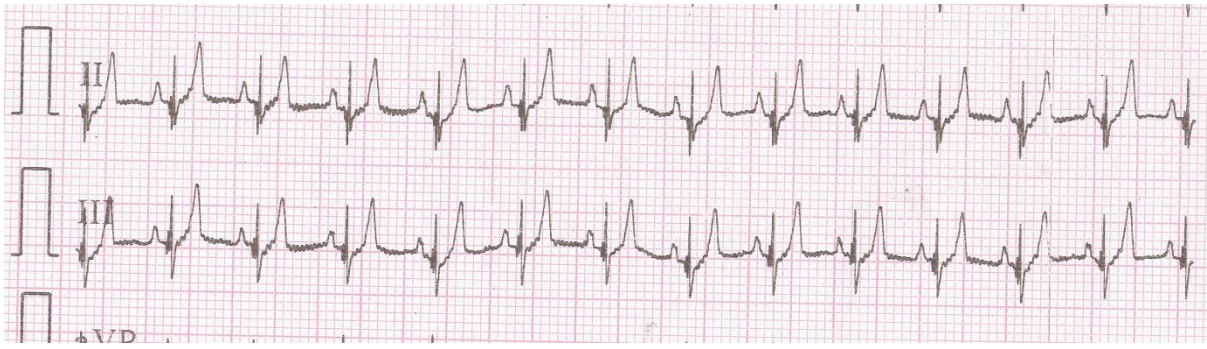


Fig. 19: ECG: Tall T waves, in this ECG, taller than the preceding R waves

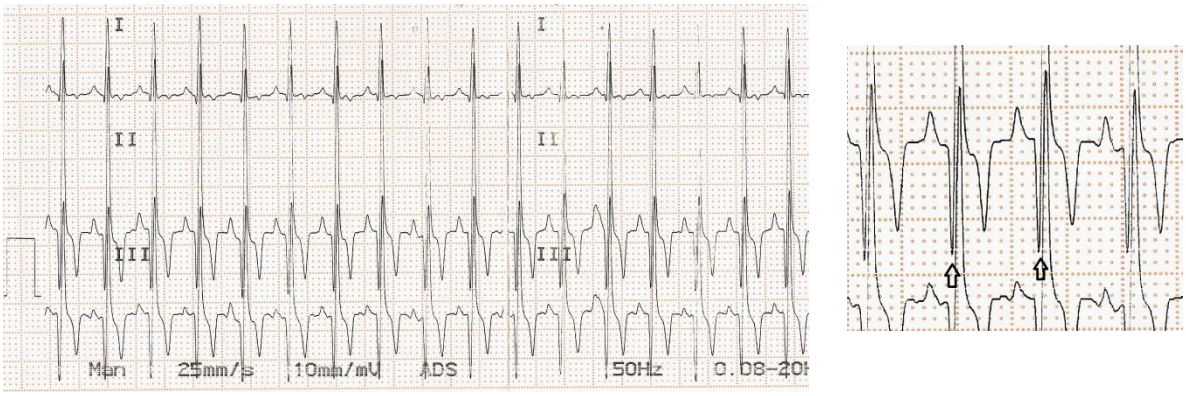


Fig. 20: ECG: Sinus tachycardia (heart rate 195 bpm) with tall R (2.9 mV) and deep Q (1.1 mV) waves

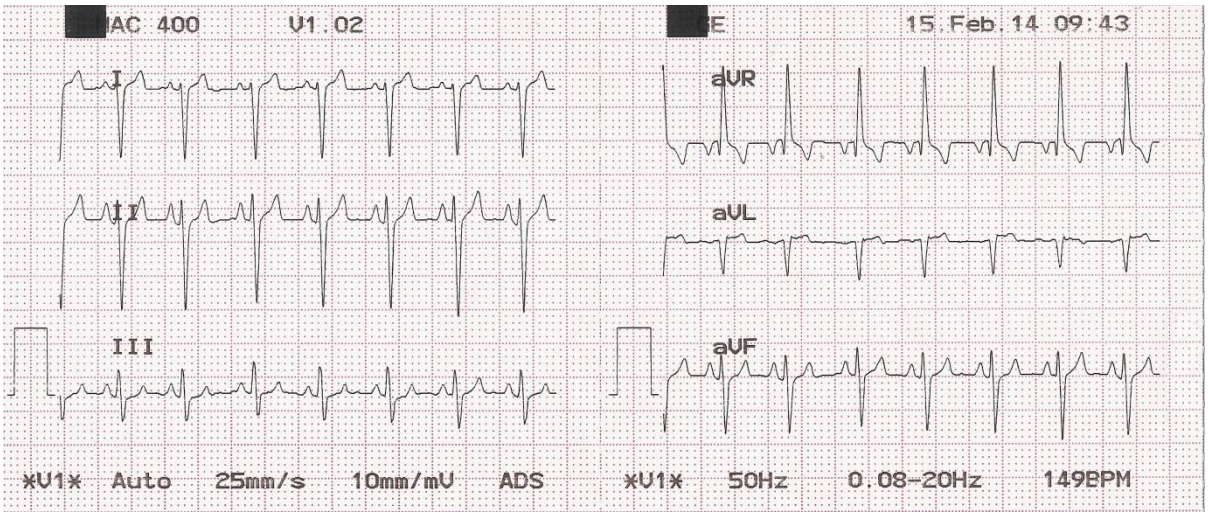


Fig. 21: ECG: Deep S waves in leads I, II, III and aVF

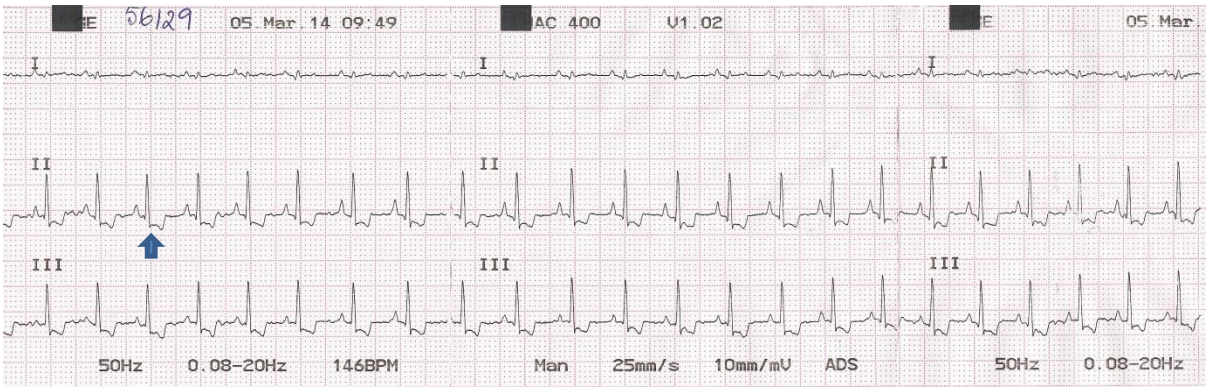


Fig.22: ECG: ST segment sagging by 0.2 mV as indicated by arrow

with obscure cardiac silhouette in 8 cases of DCM (13.56%). Ascites (3 cases, 5.1%) in DCM dogs was also diagnosed in those radiographs where the abdomen was also focussed along with the thorax.

4.6 Hematological findings in dogs with congestive heart failure

The total leukocyte count ranged from 3.5 X 10^3 cells/ µL to 29.0 X 10^3 cells/ µL and the mean ± S.E. was 12.12 ± 0.6 X 10^3 cells/ µL. The haemoglobin value ranged from 7.9 g/dL to 19.2 g/dL and the mean ± S.E. was 12.98 ± 0.27 g/dL. Total erythrocyte count ranged from 4.14 X 10^6 cells/ µL to 9.0 X 10^6 cells/ µL with the mean ± S.E. was 6.44 ± 0.11 X 10^6 cells/ µL. The packed cell volume ranged from 24.1 % to 58.5% and the mean ± S.E. was 41.69 ± 0.73 %. The platelet count ranged from 0.42 X 10^5 cells/ µL to 8.59 X 10^5 cells/ µL and the mean ± S.E. was 2.75 ± 0.14 X 10^5 cells/ µL. The same is depicted in Table 12.

4.7 Blood biochemistry values in dogs with congestive heart failure

4.7.1 Routine biochemical evaluation

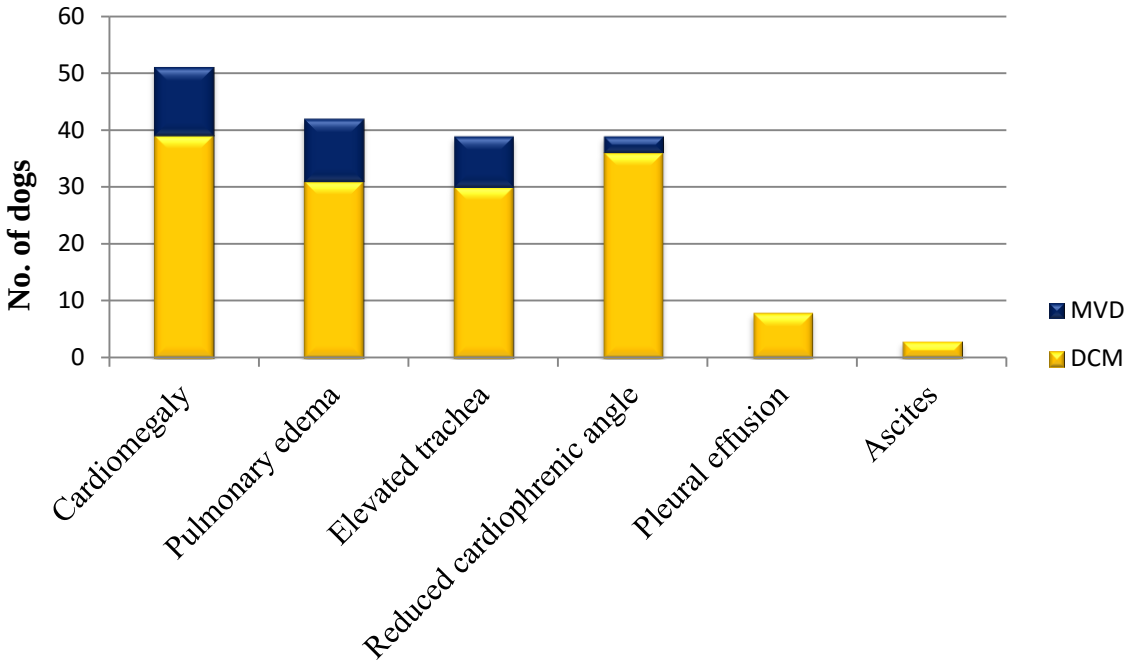
The plasma creatinine values ranged from 0.4 to 2.1 mg/dL and the mean ± S.E. was 1.03 ± 0.03 mg/dL. The plasma alanine aminotransferase values ranged from 7.0 to 98 U/L and the mean ± S.E. was 35.81 ± 2.34 U/L. The serum proteins ranged from 4.9 to 8.6 g/dL and the mean ± S.E. was 6.25 ± 0.1 g/dL. The serum albumin ranged from 2.0 to 4.0 g/dL and the mean ± S.E. was 2.65 ± 0.04 g/dL. The serum sodium values ranged from 123 to 161 mg/dL and the mean ± S. E. was 142.2 ± 0.94 mg/dL. The serum potassium values ranged from 3 to 6 mg/dL and the mean ± S. E. was 4.28 ± 0.10 mg/dL. These findings are depicted in Table 12.

Table 11: Radiographic findings in CHF due to DCM and MVD in dogs (n=59)

Abnormal findings	No. of dogs		Total
	DCM	MVD	
Cardiomegaly	39	12	51(86.44)
Pulmonary edema	31	11	42 (71.18)
Elevated trachea	30	9	39 (66.1)
Reduced cardiophrenic angle	36	3	39 (66.1)
Pleural effusion	8	0	8 (13.56)
Ascites	3	0	3 (5.1)

Note: Figures in parentheses indicate percentage

Fig. 23: Radiographic findings in CHF due to DCM and MVD in dogs (n=59)



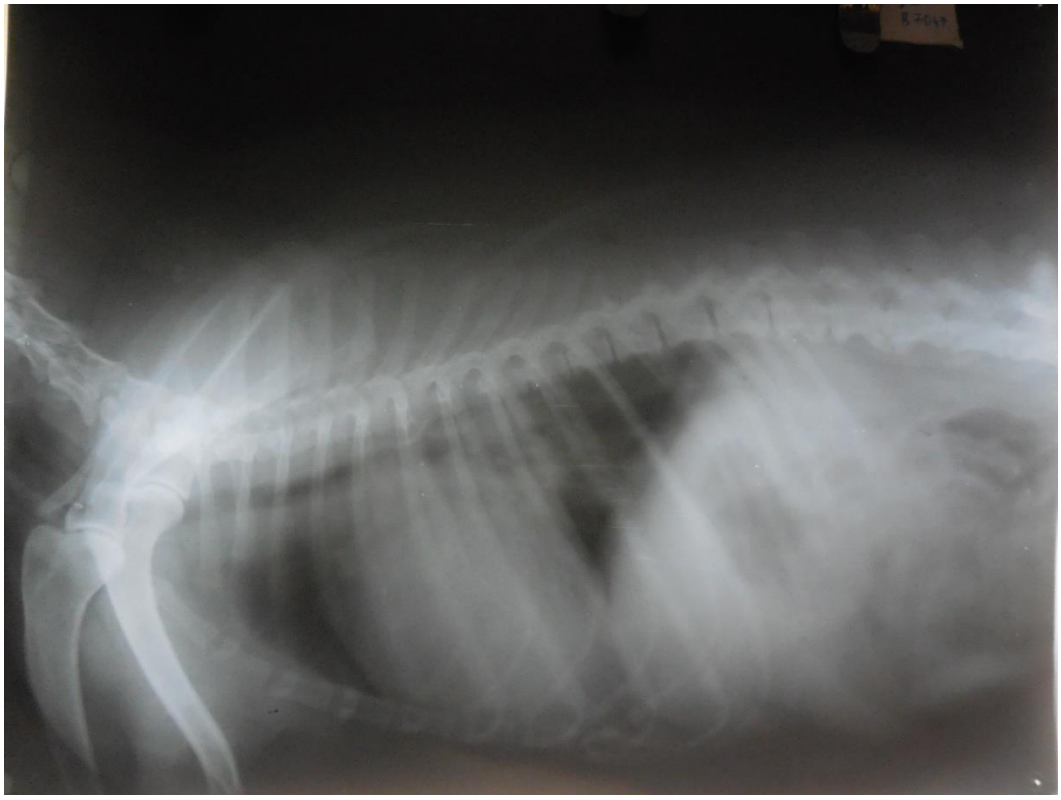


Fig. 24: Thoracic radiograph: Cardiomegaly (heart occupies 4 intercostal spaces)

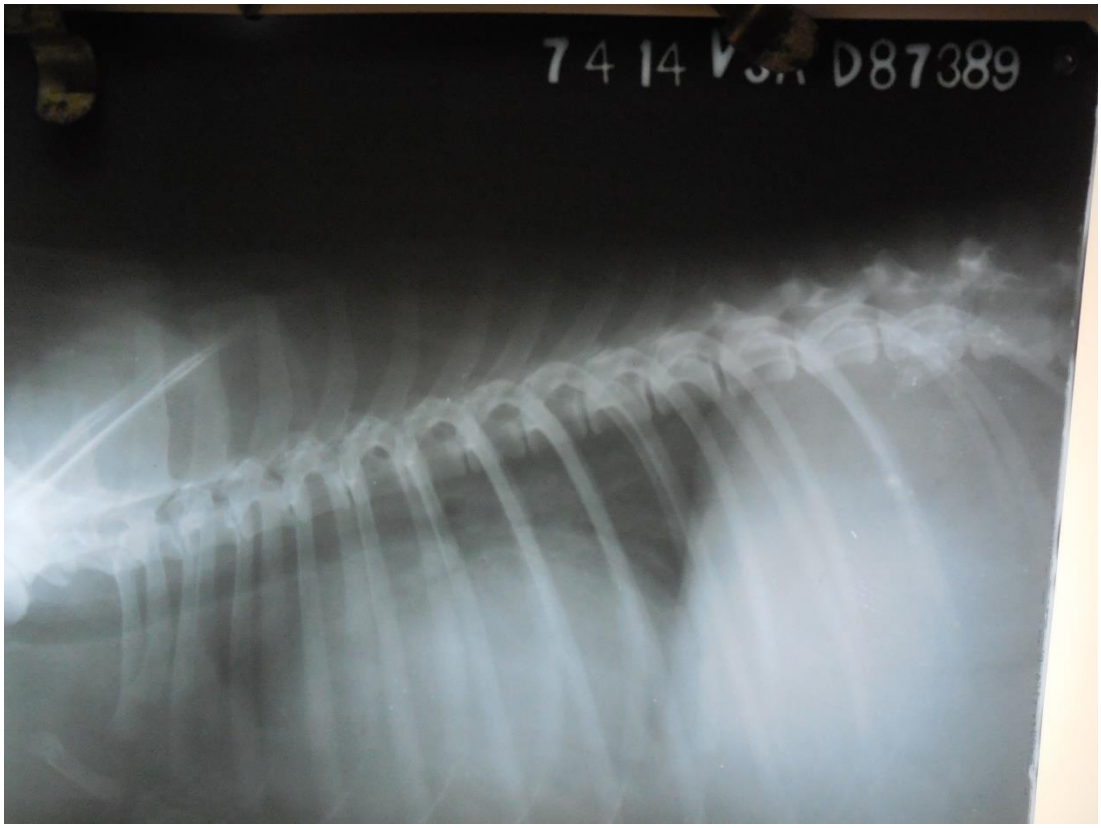


Fig. 25: Thoracic radiograph: Severe cardiomegaly (compare with Fig. 24)

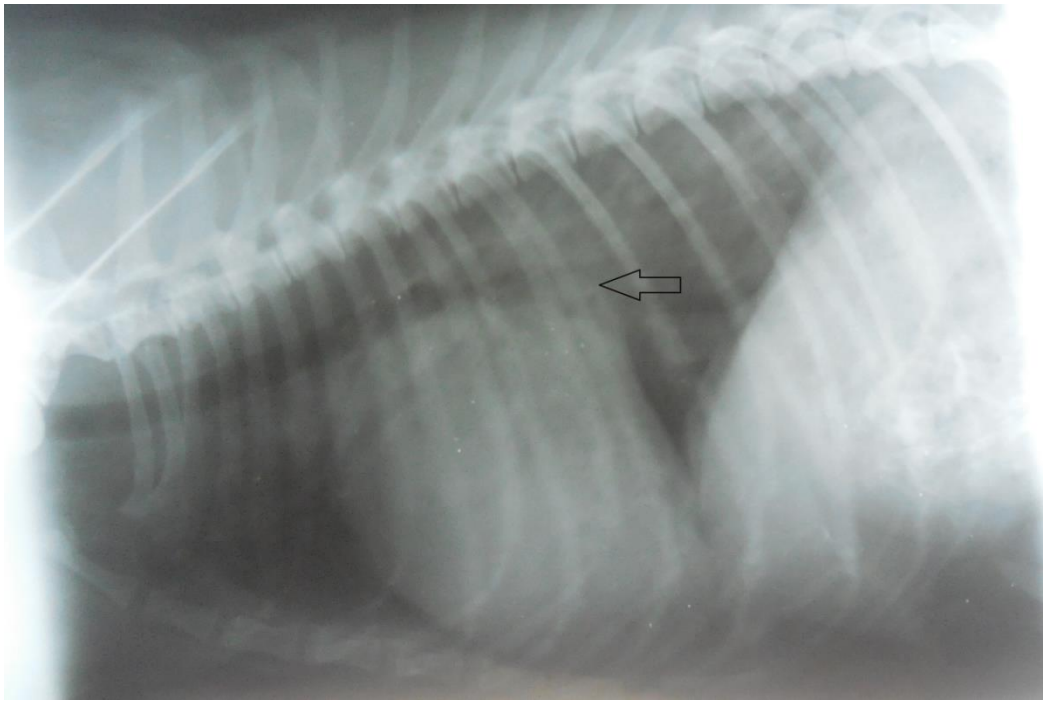


Fig. 26: Thoracic radiograph: Left atrial enlargement (arrow) and hypervascularisation of lungs

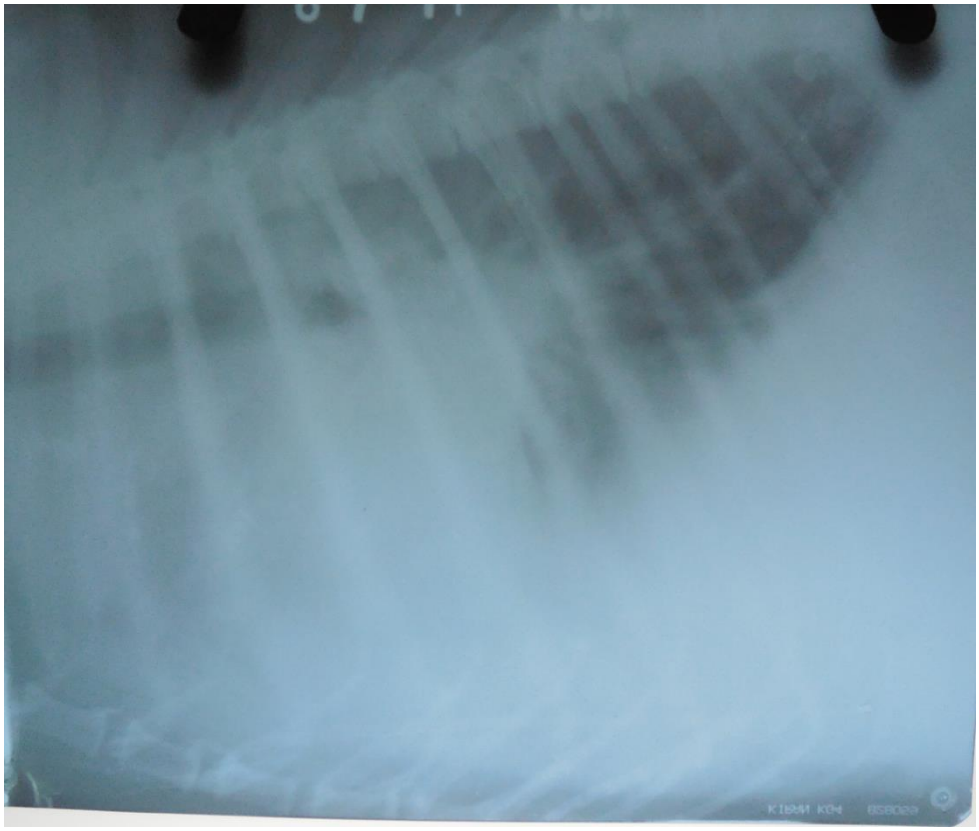


Fig. 27: Thoracic radiograph: Pleural effusion obscuring cardiac silhouette, but not the caudal lung field

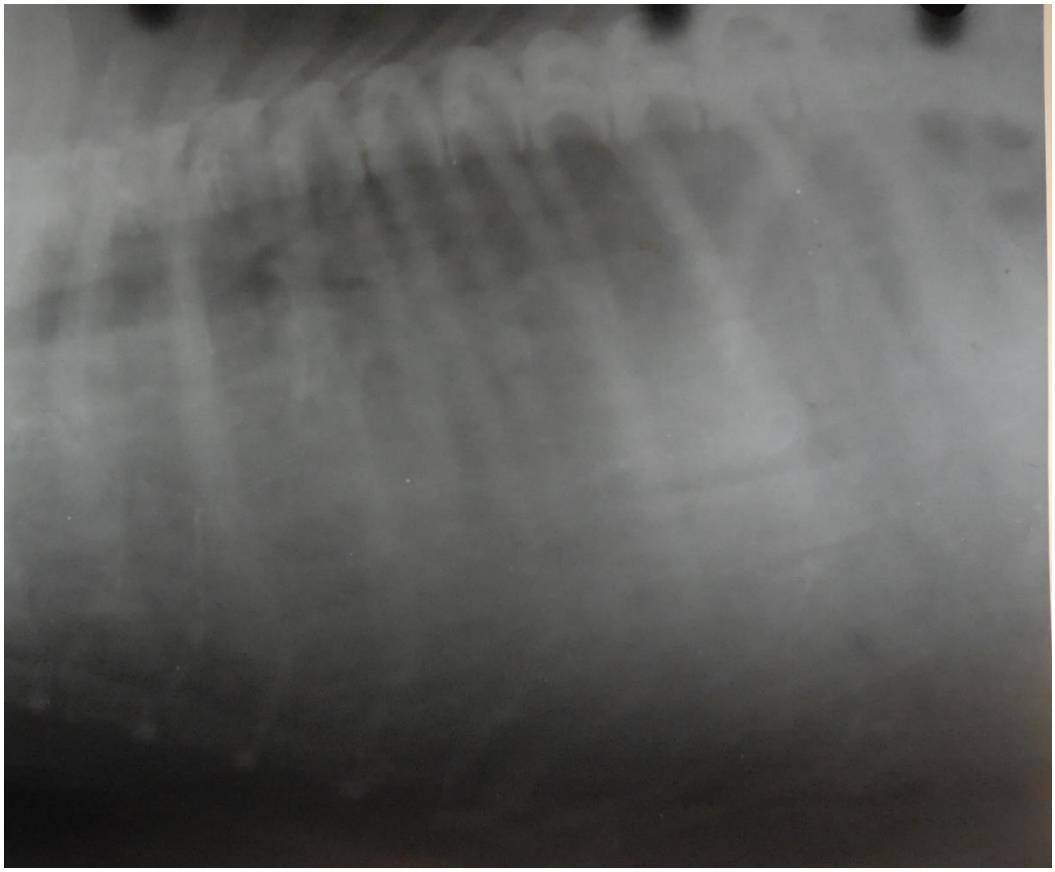


Fig.28: Thoracic radiograph: Pleural effusion obscuring both cardiac and lung fields (elevated trachea can be discerned)

Table 12: Hematological and routine biochemical test results in CHF dogs (n=78)

Parameter	**Range**	**Mean ± S. E.**
TLC (x 10^3 cell/µL)	3.5 - 29.0	12.12 ± 0.6
TEC (x 10^6 cell/µL)	4.14-9.0	6.44 ± 0.11
Hemoglobin (g/dL)	7.9-19.2	12.98 ± 0.27
PCV (%)	24.1-58.5	41.69 ± 0.73
Platelet (x 10^5/µL)	0.42-8.59	2.75 ± 0.14
Plasma creatinine (mg/dL)	0.4-2.1	1.03 ± 0.03
Plasma ALT (U/L)	7.0-98	35.81 ± 2.34
Total serum protein (g/dL)	4.9-8.6	6.25 ± 0.1
Serum albumin (g/dL)	2.0 -4.0	2.65 ± 0.04
Serum sodium (mg/dL)	123 -161	142.2 ± 0.94
Serum potassium (mg/dL)	3-6	4.28 ± 0.10

4.7.2 Cardiac biomarkers

Three cardiac biomarkers, CK-MB, cTnI and NT-proBNP were measured to analyse their utility in the diagnosis of cardiac disease as compared to the conventional tests like electrocardiography, radiography and echocardiography.

4.7.2.1 Serum CK-MB

CK-MB was analysed from 36 serum samples collected from dogs suffering from congestive heart failure. The values ranged from 8 to 25 U/L and the mean ± S. E. was 15.86 ± 0.66 U/L. Serum from ten normal dogs was collected to establish a normal range for the CK-MB enzyme in our laboratory and the values ranged between 6 and 17 U/L and the mean ± S. E. was 12.3 ± 1.1 U/L. Only 12 dogs with CHF had a value of more than 17 U/L which was the highest recording in normal dogs.

4.7.2.2 Cardiac troponin I (ELISA based method)

cTnI was analysed using a canine cTnI sandwich ELISA kit with the detection range of 0.156 ng/mL to 10 ng/mL. Ten serum samples from normal animals and 36 samples from cases of CHF were used to run the test. The normal animals did not have a detectable value (to be assumed to be 0 as per literature), except for one dog with cTnI value of 0.4 ng/mL. In the CHF group, the values ranged from 0 ng/mL to more than 10 ng/mL. The result of the test (colour development which was later quantified) is presented in Fig. 29.

4.7.2.3 Cardiac troponin I (human immunochromatography kit)

A human qualitative immunochromatography kit with an analytical sensitivity of 0.5 ng/ mL was employed to detect the presence of cTnI in dogs suffering from CHF. The

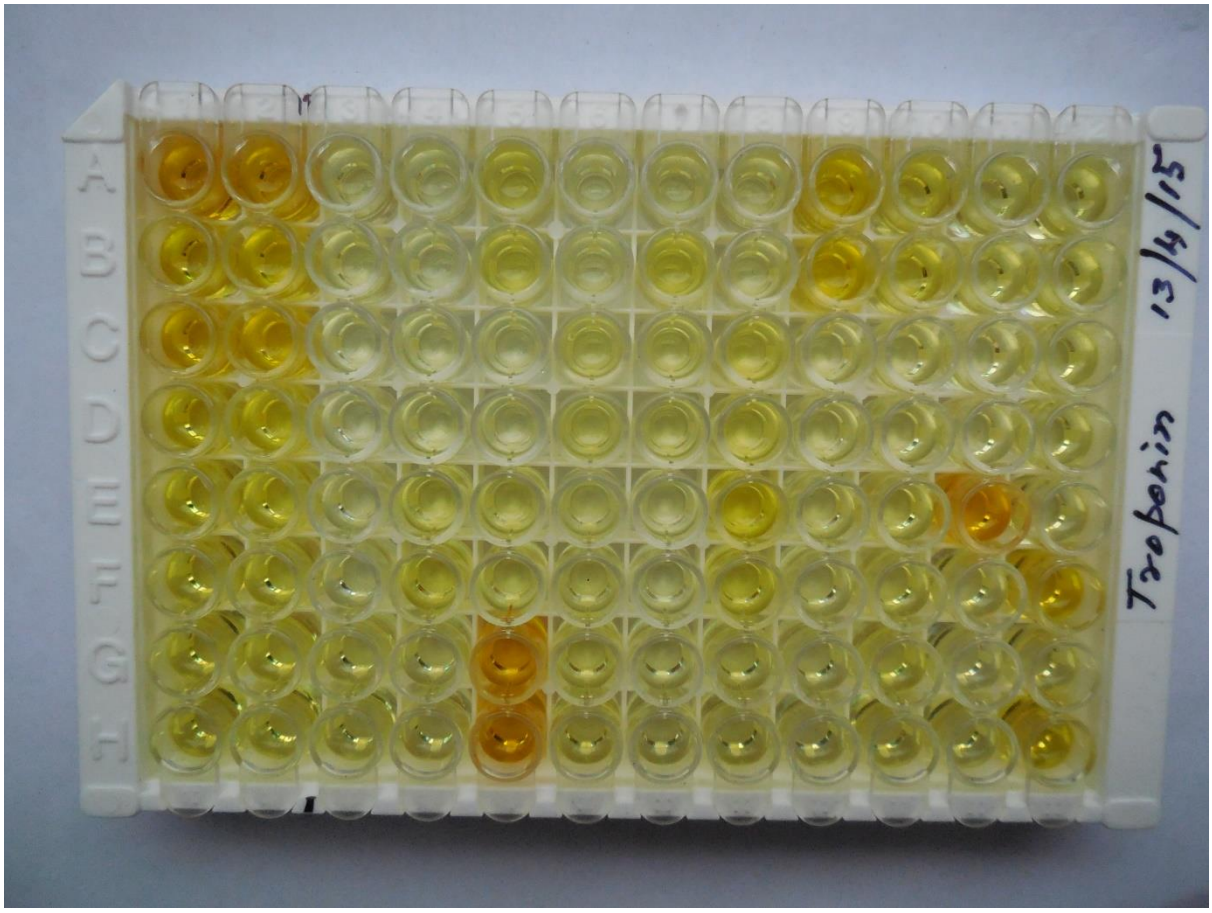


Fig. 29: ELISA plate of canine cTnI kit showing colour development after addition of stop solution

kit was validated using different concentrations of canine cTnI obtained from the same manufacturers as the canine cTnI sandwich ELISA kit. Even though the test had an analytical sensitivity of 0.5 ng/mL, in the present study the test was able to detect canine cTnI as low as 0.156 ng/mL. Out of 36 samples from CHF dogs, the kit was able to detect cTnI presence in 19 samples and the reaction ranged from strongly positive (thick, dark line) to weakly positive (thin, pale line). The sample from a normal dog with 0.4 ng/mL of cTnI was also positive by this kit. Figs. 30 (a-d) depict the results of this test.

4.7.2.4 NT-proBNP

NT-proBNP was analysed using a canine NT-proBNP sandwich ELISA kit with the detection range of 0.312 ng/mL to 20 ng/mL (30 pmol/L to 1896 pmol/L). Ten serum samples from normal animals and 36 samples from cases of CHF were used to run the test. The normal animals did not have a detectable value (to be assumed to be 0 or < 0.312 ng/mL), except for two dogs with values of 0.54 ng/mL (52 pmol/L) and 0.62 ng/mL (58 pmol/L). In the CHF group, the values ranged from undetectable to 1449 ng/mL. Fig. 31 depicts the ELISA test result of this biomarker.

4.7.2.5 Comparison of different cardiac biomarkers

There was no correlation between CK-MB with either cTnI or NT-proBNP .

Two samples had both high (or detectable) cTnI and BNP, 2.072 ng/mL and 58 pmol/L in one dog and > 10 ng/mL and 605 pmol/L in another respectively. cTnI and NT-proBNP together could diagnose 10 cases of CHF.

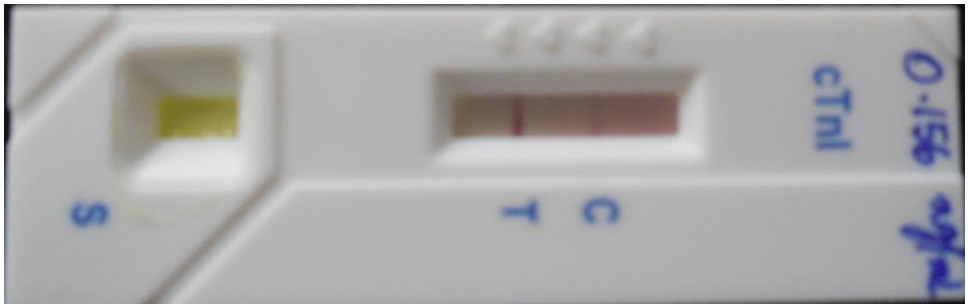


Fig. 30: a: Human immunochromatography kit validated for canine cTnI showing a strongly positive band in "T" (test) slot.

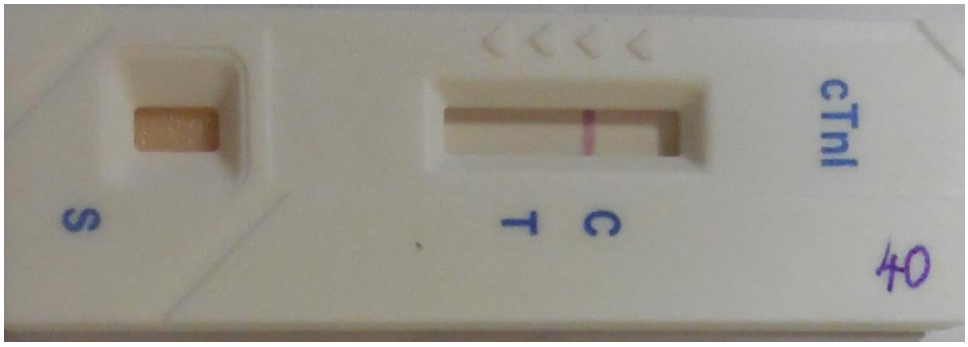


Fig. 30b: A sample negative for cTnI as indicated by a band only in the "C" (control) slot

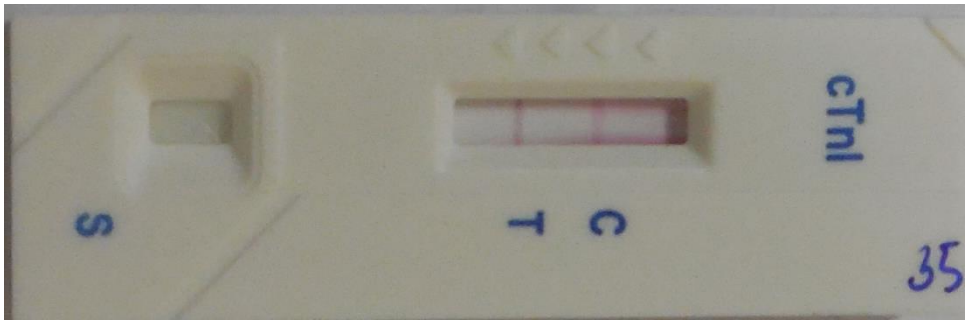


Fig. 30c: A sample from a CHF dog showing strong positive result

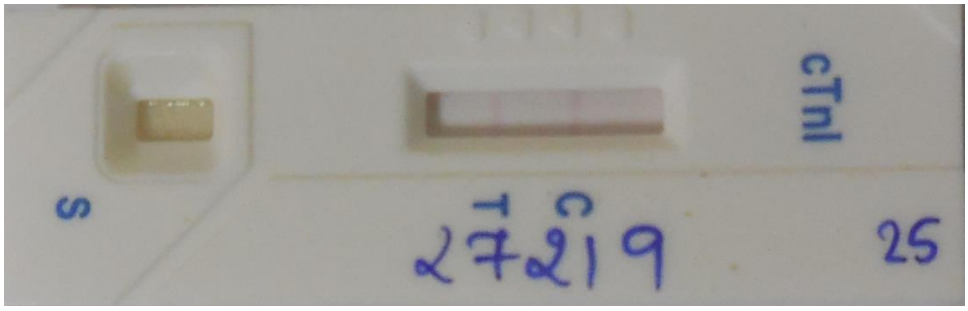


Fig. 30d: A sample from a CHF dog showing weak positive result

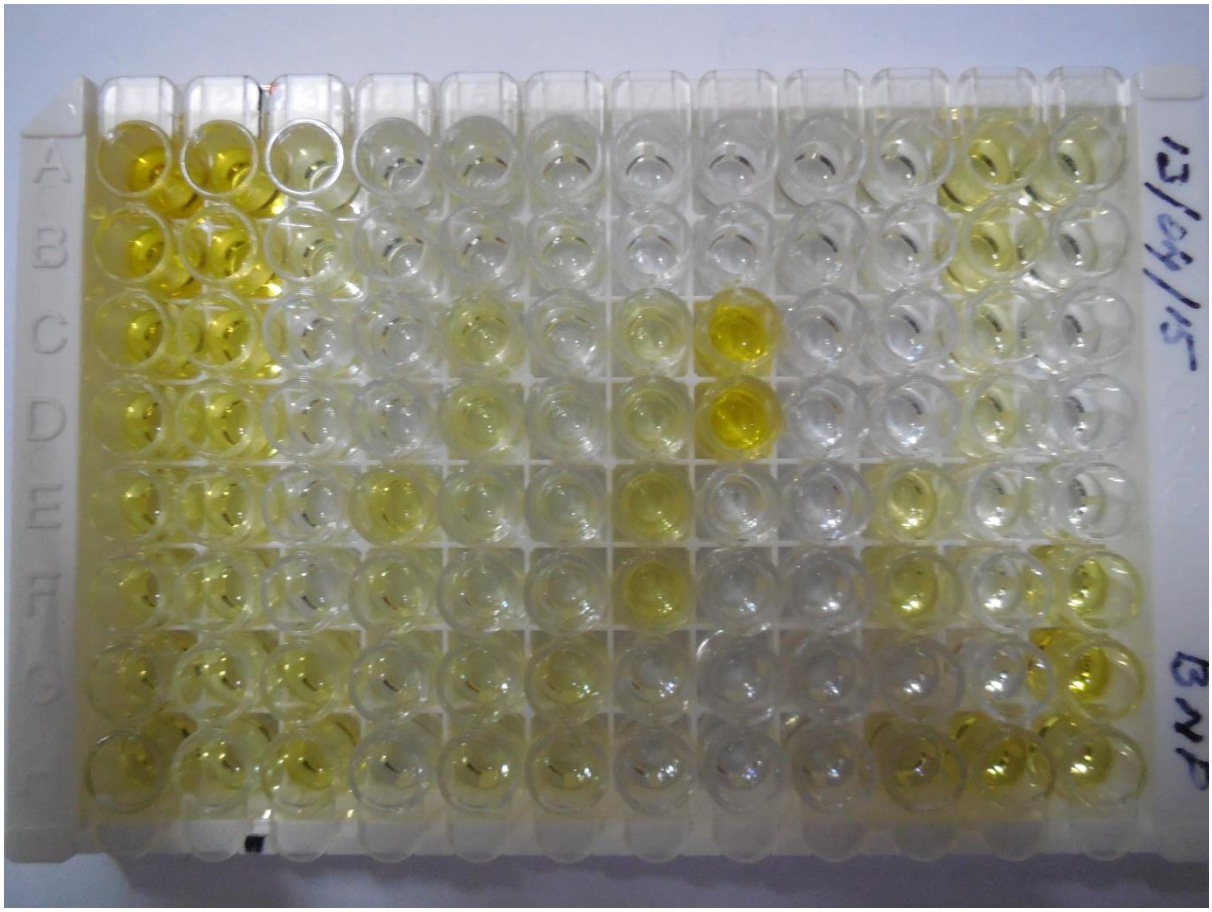


Fig. 31: ELISA plate of canine NT-proBNP kit showing colour development after addition of stop solution

4.8 Echocardiography in dogs with congestive heart failure

Echocardiographic examination of dogs with congestive heart failure revealed that out of the 78 cases, 59 dogs suffered from DCM and 19 dogs had MVD. DCM was diagnosed by decrease in the fractional shortening and increase in E point to septal separation in M-mode and decrease in ejection fraction in 2-D mode. MVD was diagnosed by the presence of mitral regurgitation and enlarged left atrium along with normal to high systolic function indices (EF and FS). Tables 13, 14 and Figs. 33 to 43 depict the following results.

The range and mean ± S. E. of echocardiographic parameters in M-mode in CHF dogs with DCM were as follows: IVS_d 0.44 to 1.32 cm and 0.95 ± 0.02 cm, $LVID_d$ 2.12 to 7.91 cm and 4.51 ± 0.13 cm, LVFWd 0.73 to 1.76 and 1.06 ± 0.02 cm, IVS_s 0.53 to 1.51 and 1.05 ± 0.02 cm, $LVID_s$ 1.63 to 7.03 cm and 3.88 ± 0.12 cm, $LVFW_s$ 0.85 to 2.29 cm and 1.22 ± 0.03 cm and fractional shortening 6.56 to 22.86 and 14.06 ± 0.55 % respectively. The range and mean ± S. E. of echocardiographic parameters in 2-D mode in CHF dogs with DCM were as follows: EPSS 0.79 to 1.51 cm and 1.11 ± 0.02 cm, LVL_d 4.46 to 9.16 cm and 5.87 ± 0.32 cm, LVL_s 3.6 to 8.57 cm and 5.27 ± 1.29 cm, EDV 16.5 to 326 ml and 81.67 ± 19.78 ml, ESV 10.53 to 256.9 ml and 61.97 ± 15.86 ml and ejection fraction 14.46 to 39.33 % and 26.51 ± 1.78 % respectively. The ESV was indexed to body surface area to study the systolic dysfunction. The ESVI ranged from 25.82 to 169 mL/m^2 and the mean ± S. E. was 56.52 ± 10.69 mL/m^2.

The range and mean ± S. E. of echocardiographic parameters in M-mode in CHF dogs with MVD were as follows: IVS_d 0.59 to 1.14 cm and 0.85 ± 0.03 cm, $LVID_d$ 2.15 to 4.75 cm and 3.31 ± 0.15 cm, LVFWd 0.74 to 2.43 and 1.21 ± 0.10 cm, IVS_s 0.74 to 1.69

and 1.23 ± 0.07 cm, $LVID_s$ 1.11 to 2.55 cm and 1.94 ± 0.10 cm, $LVFW_s$ 0.99 to 2.53 cm and 1.68 ± 0.09 cm and fractional shortening 31.11 to 56 and 41.18 ± 1.60 % respectively. The range and mean ± S. E. of echocardiographic parameters in 2-D mode in CHF dogs with MVD were as follows: EPSS 0.28 to 0.71 cm and 0.44 ± 0.03 cm, LVL_d 2.09 to 5.04 cm and 3.61 ± 0.52 cm, LVL_s 1.67 to 4.16 cm and 2.97 ± 0.4 cm, EDV 3.45 to 52.09 ml and 17.27 ± 9.07 ml, ESV 1.25 to 21.6 ml and 6.94 ± 3.76 ml and ejection fraction 51.89 to 64.44 % and 59.11 ± 2.63 % respectively. The left atrial diameter in right parasternal 2D short axis view ranged from 2.08 cm to 3.61 cm with 2.92 ± 0.13 as the mean and standard error. The aortic diameter in the same views ranged from 1.09 cm to 1.77 cm with the mean 1.48 ± 0.04 cm. The LA:Ao ratio ranged from 1.71 to 2.32 with 1.96 ± 0.04 as the mean. Mitral regurgitation as observed from colour flow Doppler was severe in 3 dogs and moderate in 16 dogs and mitral valve thickening (degeneration) was appreciated in 13 cases.

Pleural effusion (8) and ascites (25) could also be confirmed in the dogs suffering from CHF by ultrasonography.

4.9 Prognostic potential of cardiac biomarkers

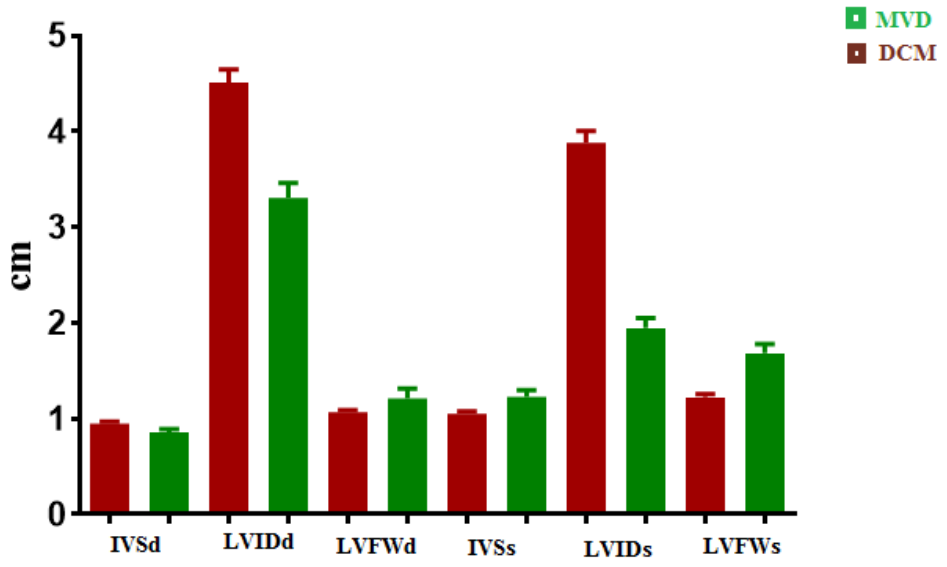
4.9.1. Cardiac biomarkers and clinical signs

All dogs with high cTnI and NT-proBNP had bilateral/ biventricular heart failure with signs of cough and ascites. One dog also had orthopnoea and epistaxis/ hemoptysis. All these dogs except one had atrial fibrillation.

Table 13: Echocardiographic findings in M-mode in CHF dogs (n=78)

Parameter	**DCM**		**MVD**	
	Range	**Mean ± S. E.**	**Range**	**Mean ± S. E.**
IVS_d (cm)	0.44 to 1.32	0.95 ± 0.02	0.59 to 1.14	0.85 ± 0.03
$LVID_d$ (cm)	2.12 to 7.91	4.51 ± 0.13	2.15 to 4.75	3.31 ± 0.15
$LVFW_d$ (cm)	0.73 to 1.76	1.06 ± 0.02	0.74 to 2.43	1.21 ± 0.10
IVS_s (cm)	0.53 to 1.51	1.05 ± 0.02	0.74 to 1.69	1.23 ± 0.07
$LVID_s$ (cm)	1.63 to 7.03	3.88 ± 0.12	1.11 to 2.55	1.94 ± 0.10
$LVFW_s$ (cm)	0.85 to 2.29	1.22 ± 0.03	0.99 to 2.53	31.11 to 56
Fractional shortening (%)	6.56 to 22.86	14.06 ± 0.55	31.11 to 56	41.18 ± 1.60

Fig. 32: Echocardiographic fingings in M-mode in CHF dogs (n=78)

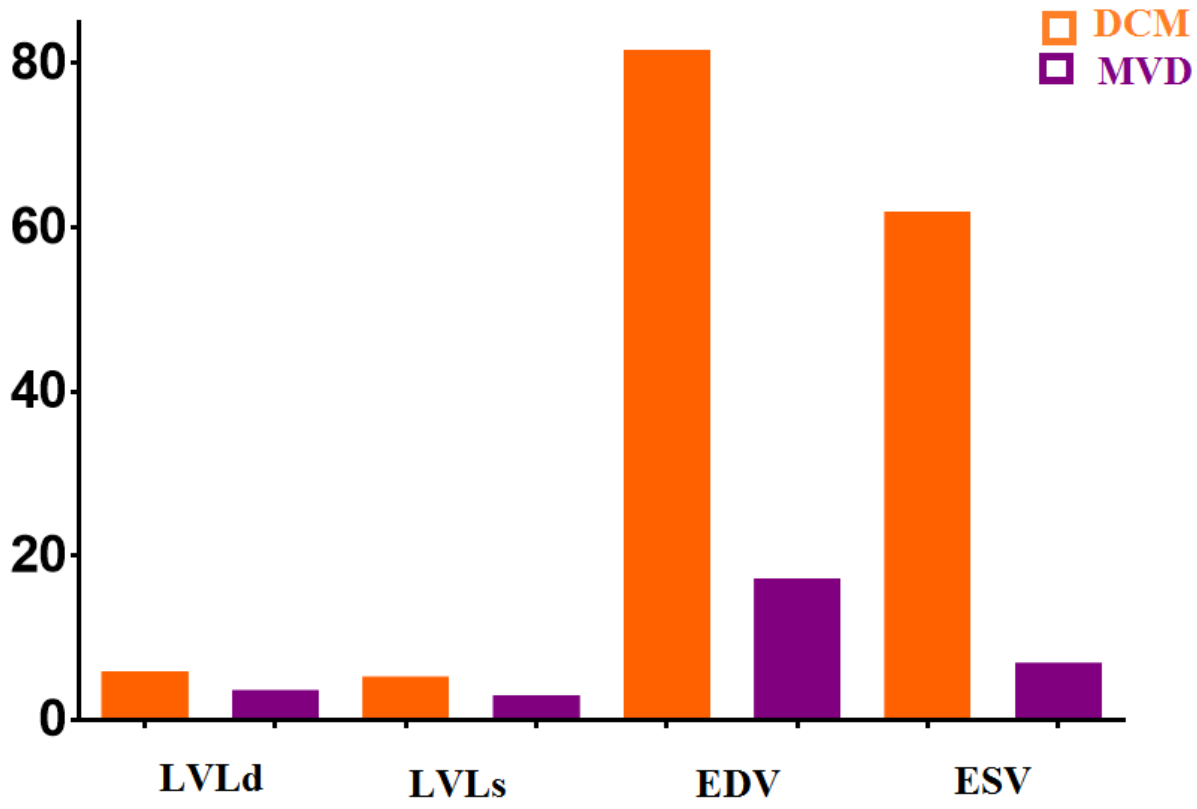


IVS: Interventricular septum
LVID: Left ventricular internal diameter
LVFW: Left ventricular free wall
s: systole
d: diastole

Table 14: Echocardiographic findings in 2D-mode in CHF dogs (n=78)

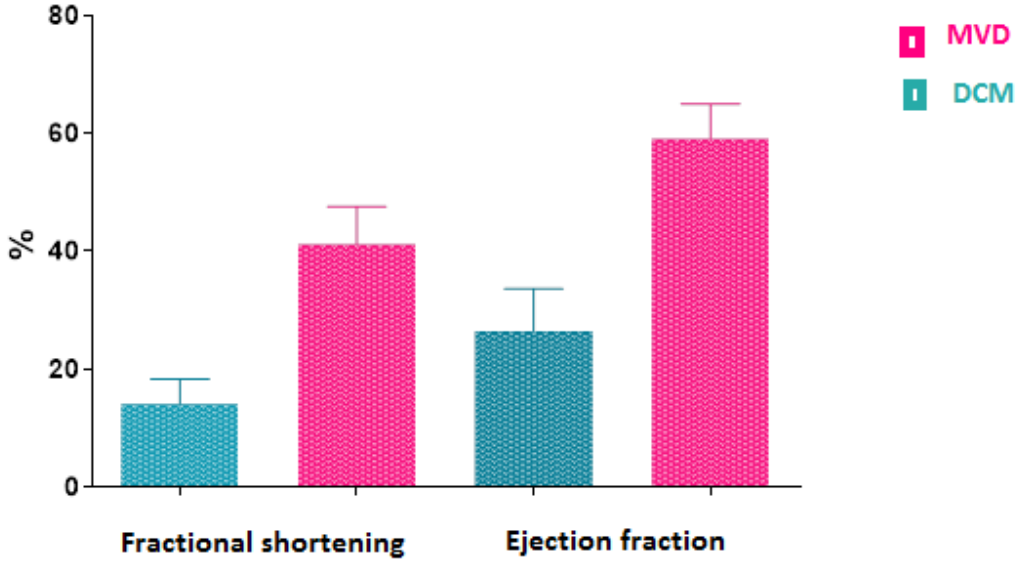
Parameter	DCM		MVD	
	Range	**Mean ± S. E.**	**Range**	**Mean ± S. E.**
EPSS (cm)	0.79 to 1.51	1.11 ± 0.02	0.28 to 0.71	0.44 ± 0.03
LVL_d (cm)	4.46 to 9.16	5.87 ± 0.32	2.09 to 5.04	3.61 ± 0.52
LVL_s (cm)	3.6 to 8.57	5.27 ± 1.29	1.67 to 4.160	2.97 ± 0.4
EDV (mL)	16.5 to 326	81.67 ± 19.78	3.45 to 52.09	17.27 ± 9.07
ESV (mL)	10.53 to 256.9	61.97 ± 15.86	1.25 to 21.6	6.94 ± 3.76
Ejection fraction (%)	14.46 to 39.33	26.51 ± 1.78	51.89 to 64.44	59.11 ± 2.63

Fig. 33: Echocardiographic findings in 2D-mode in CHF dogs (n=78)



LVL: Left ventricular length in cm
EDV: End distolic volume in mL
ESV: End systolic volume in mL
d: diastole
s: systole

Fig. 34: Systolic function in DCM and MVD dogs (n=78)



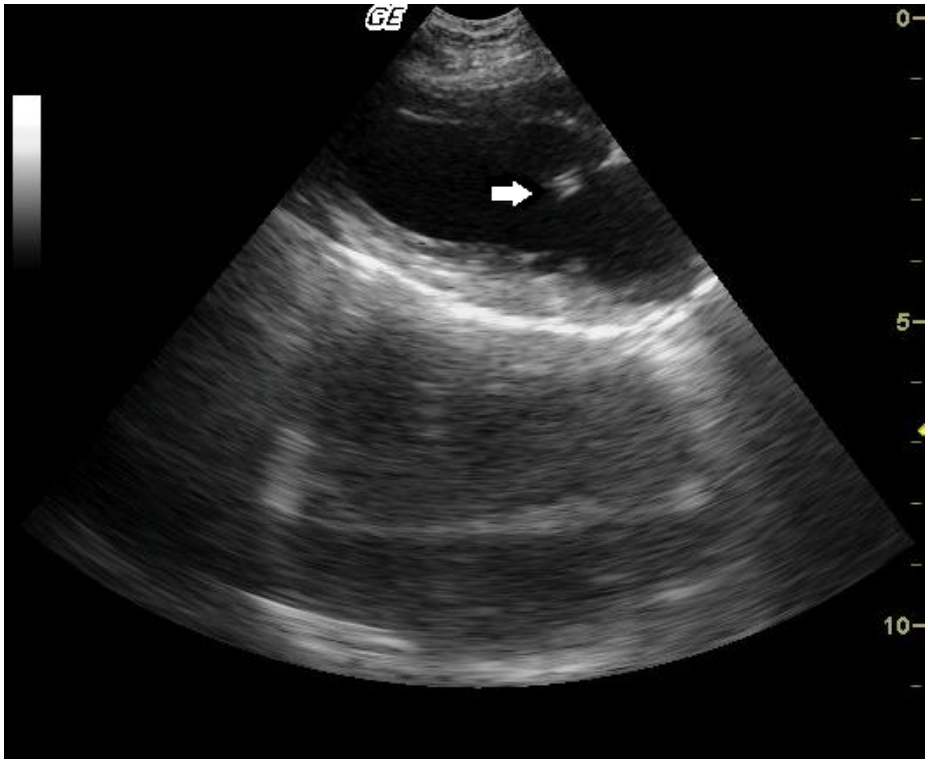


Fig. 35: Right parasternal 2D long axis echocardiography view indicating the presence of anterior mitral leaflet degeneration (arrow)

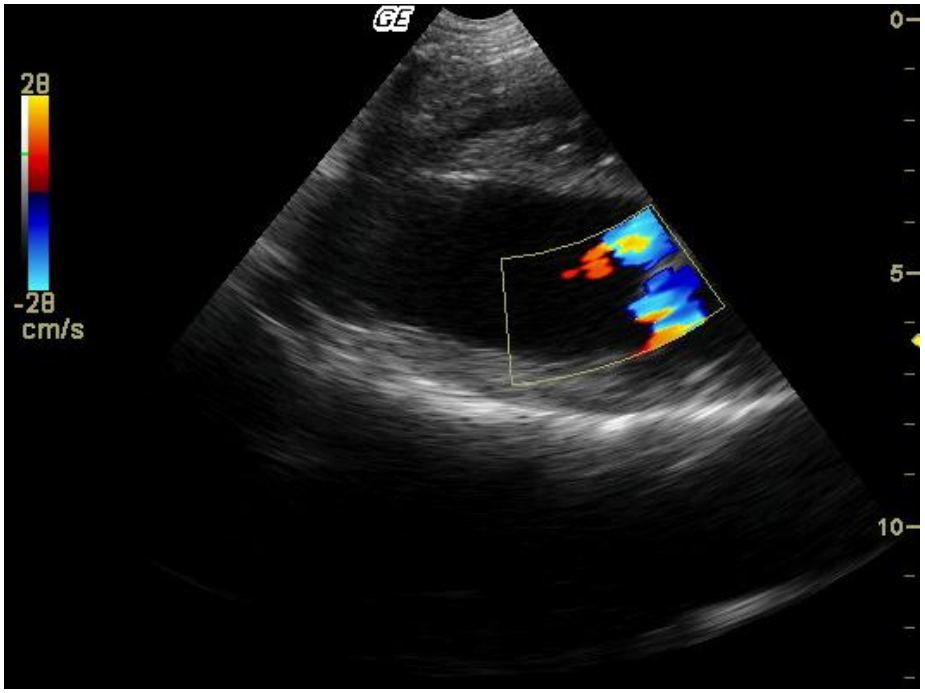


Fig. 36: Right parasternal 2D long axis echocardiography view indicating moderate mitral regurgitation by colour flow Doppler

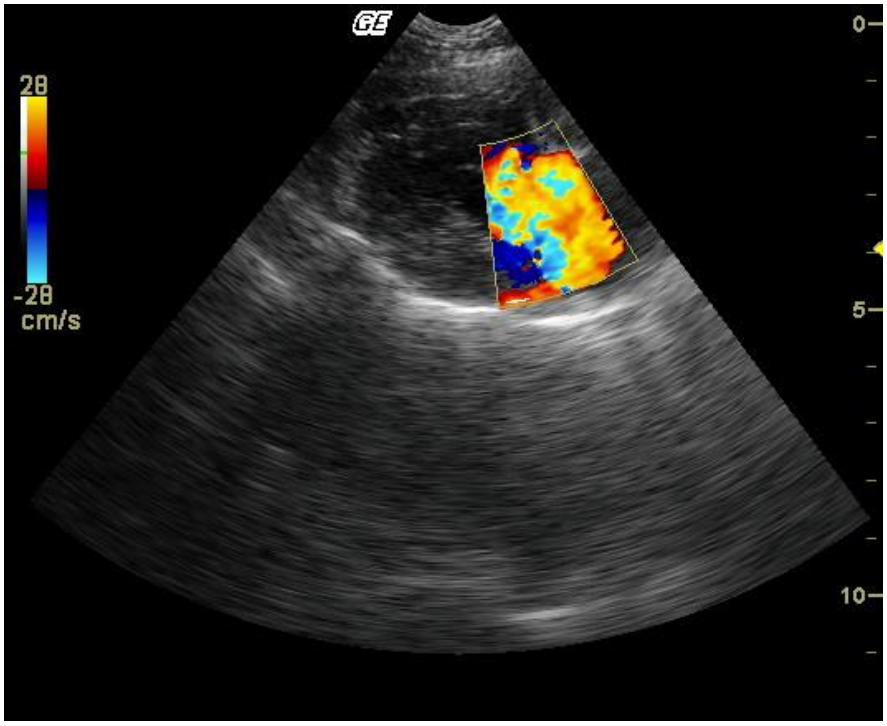


Fig. 37: Right parasternal 2D long axis echocardiography view indicating severe mitral regurgitation (occupying the entire left atrium) by colour flow Doppler

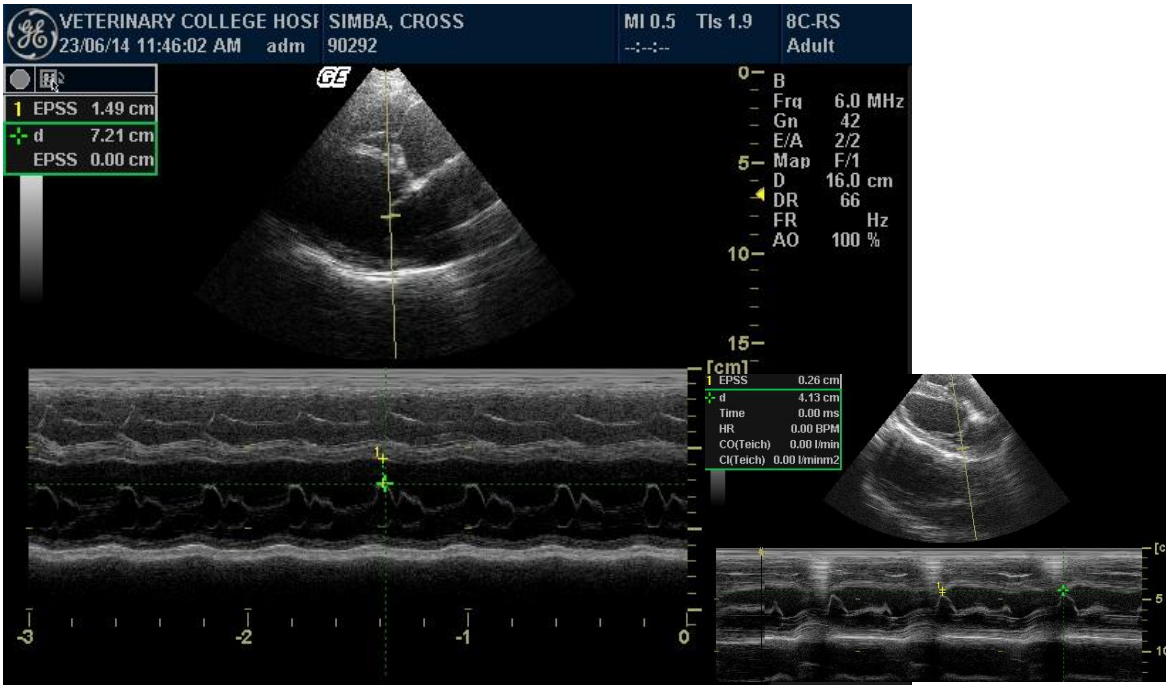


Fig. 38: Right parasternal M-mode short axis echocardiography view indicating increased EPSS in a dog with DCM (Inset: A view from a normal dog with normal EPSS)

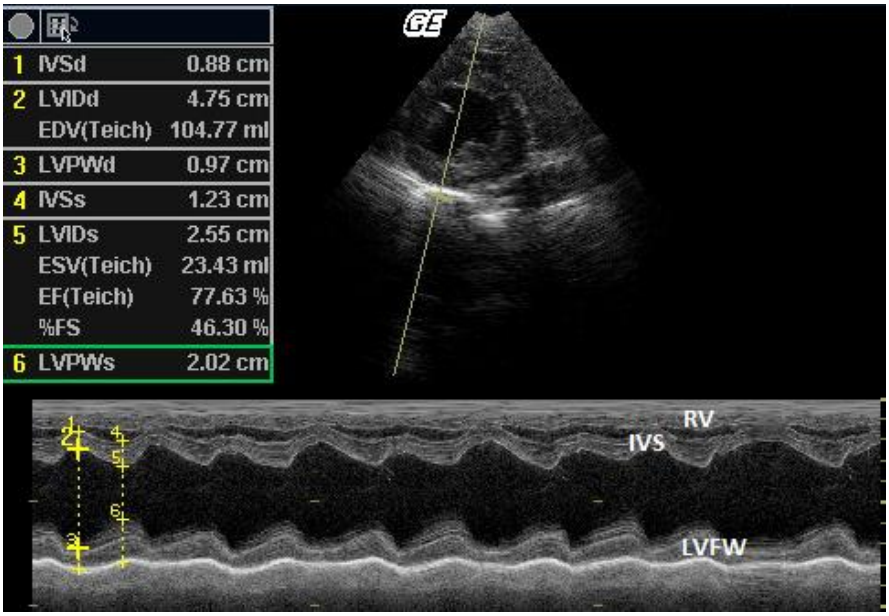


Fig. 39: a: Right parasternal M-mode short axis echocardiography view at the level of the papillary muscles indicating high normal FS in a MVD dog (Note increased excursion of interventricular septum and the left ventricular free wall)

RV: right ventricular wall, IVS: interventricular septum, LVFW: left ventricular free wall

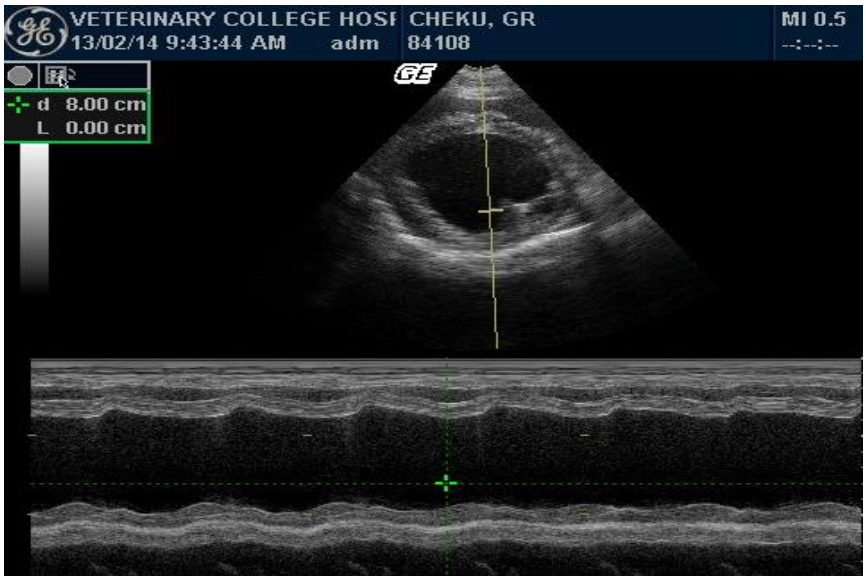


Fig. 39 b: Right parasternal M-mode short axis echocardiography view at the level of the papillary muscles indicating decreased FS in a DCM dog (Note decreased excursion of the interventricular septum and the left ventricular free wall)

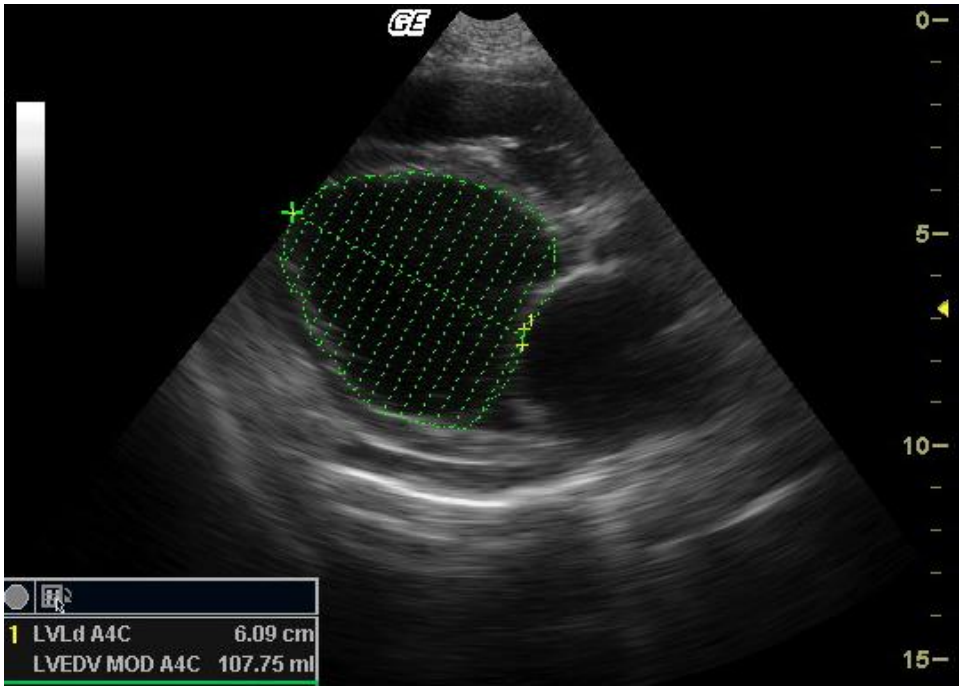


Fig. 40: Right parasternal 2D long axis echocardiography view indicating measurement of left ventricular dimensions (length and volume)

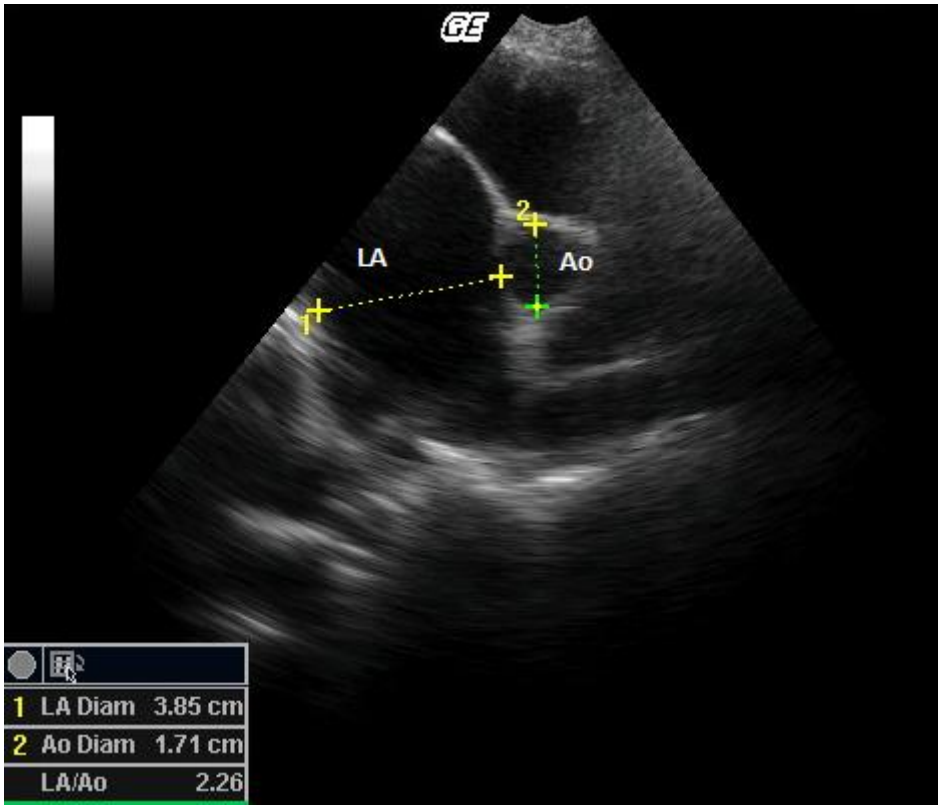


Fig. 41: Right parasternal 2D short axis echocardiography view indicating increased LA/Ao (LA: left atrial diameter, Ao: aortic diameter)

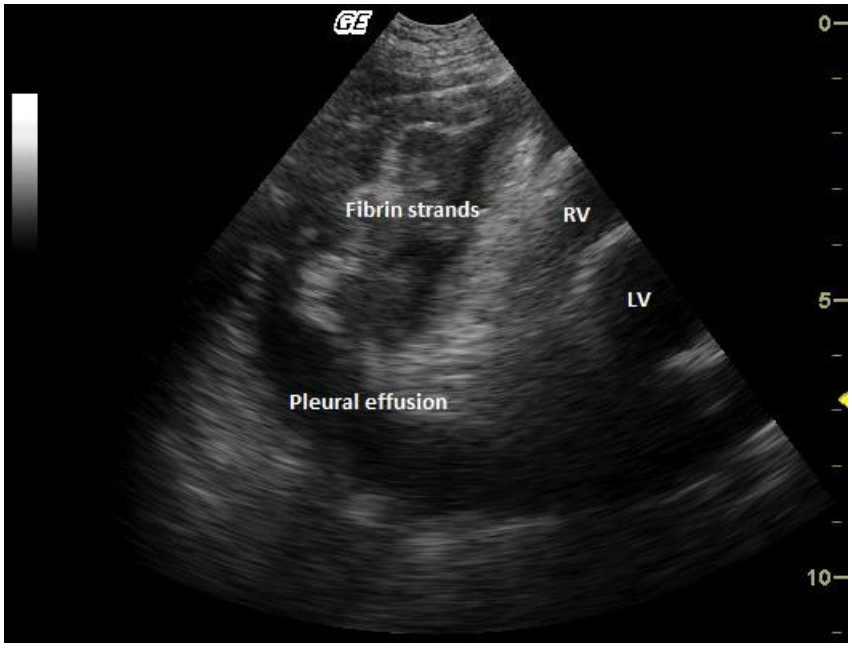


Fig. 42: Right parasternal 2D long axis echocardiography view indicating long standing pleural effusion in bilateral heart failure in DCM (note fibrin stands, RV: right ventricle, LV: left ventricle)

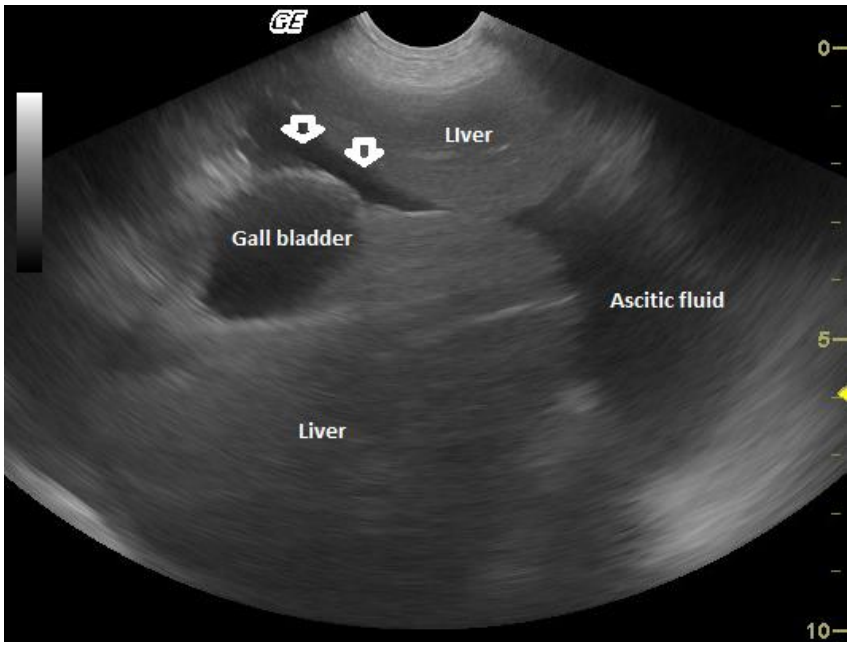


Fig. 43: Abdominal ultrasound examination revealing ascites in a case of DCM (arrows indicating easily visualised liver lobe and gall bladder margins due to the presence of free fluid in the peritoneal cavity)

4.9.2 Cardiac biomarkers and blood biochemistry

Two dogs with the highest troponin value (>10 ng/mL) had elevated creatinine values (1.5 and 1.7 mg/dL) and the dogs with high NT-proBNP value (605 and 1449 pmol/L) also had increased creatinine levels (1.5 and 2.1 mg/dL).

4.9.3 Cardiac biomarkers and echocardiography

One dog with the high troponin (>10 ng/dL) had the highest ESVI at 169 mL/m^2. All dogs with detectable and high values of the biomarkers were diagnosed as DCM.

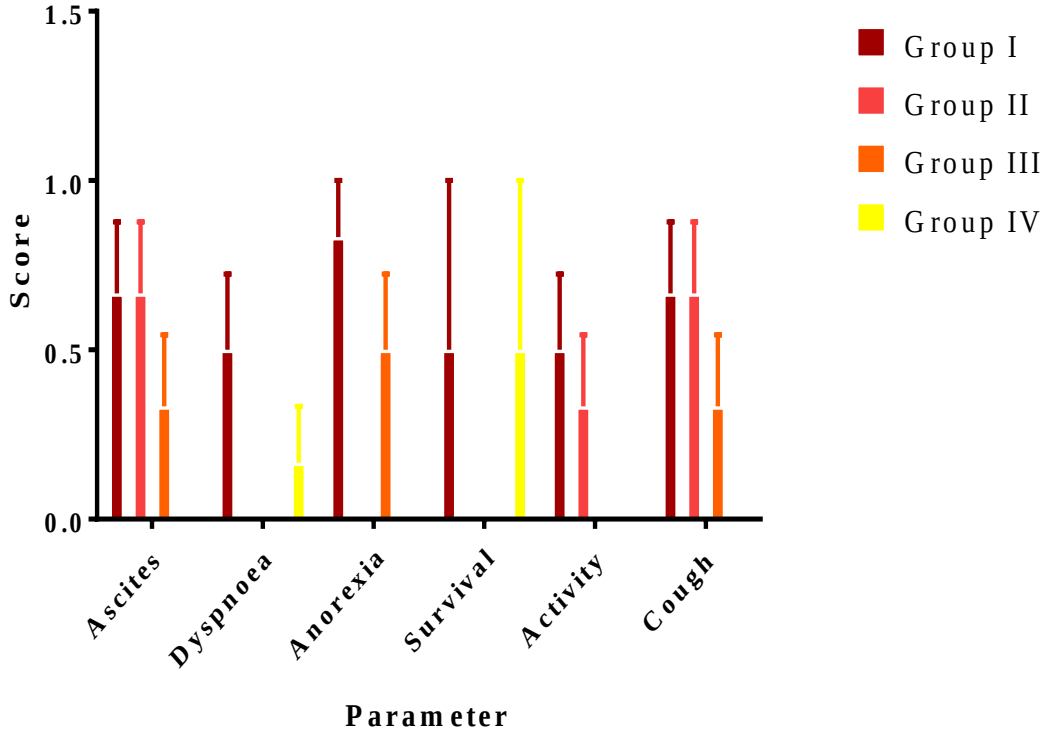
4.10 Treatment trials

The scores for the resolution of clinical signs for the four treatment groups has been depicted in Table 15 and Fig. 44 Group I had a mean score of 1.34, Group II 0.55, Group III 0.31 and Group IV 0.22. Group I had the highest score and one dog allotted to group I died during the trial period and Group IV had the lowest score despite a mortality in the group. One dog each in treatment group I and III developed azotemia at the end of one month (both dogs started out with 1.4 mg/dL at the beginning of the treatment period and at the end of one month, they had 1.6 and 1.8 mg/dL of creatinine). Three dogs had leukocyosis at the time of diagnosis (> 17000 WBCs/ µL of blood) which resolved at the end of one week of treatment without the use of any antimicrobials.

Table 15: Mean scores for resolution of clinical signs in CHF dogs

Group	**I**	**II**	**III**	**IV**
Ascites	1.33	1.33	0.67	0
Dyspnoea	1	0	0	0.33
Anorexia	2.67	0	0.5	0
Survival	1	0	0	1
Activity	1	0.67	0	0
Cough	1.33	1.33	0.67	0
Total mean	1.34	0.55	0.31	0.22

Fig. 44: Mean scores for resolution of clinical signs in CHF dogs



DISCUSSION

V. DISCUSSION

Cardiac diseases resulting in congestive heart failure are common causes of morbidity and mortality in dogs. Diagnosis is often made using information obtained from history, physical examination, routine blood tests, electrocardiography, radiography and where available echocardiography. Often, the general veterinary practitioner is faced with the difficult task of not only identifying the heart failure but also the underlying etiology/ condition that sets off numerous “maladaptive” responses in the body that eventually lead to the clinical manifestation of the disease. Only very few heart diseases can be cured, the majority have to be managed and the clinician often with access to limited diagnostic facilities and/ or with limited experience/ skills with diagnostic procedures has to arrive at the prognosis. Echocardiography has largely replaced invasive diagnostic techniques like angiocardiography in the diagnosis of heart diseases, but this facility may not always be available to all patients or veterinarians. Of late, biomarkers have been gaining importance, not only for diagnosis, but also for grading the severity of disease and for prognostication. Newer drugs like inodilators (pimobendan) are also being made available in the recent years to the veterinary cardiologist for better management of the cardiac patient. Use of complementary or alternative veterinary medicine is steadily increasing in an attempt to improve the quality of life and to prolong the life span by the administration of nutraceuticals. The results of the present study are discussed here, in which some of the newer diagnostic and therapeutic options for cardiac diseases were evaluated.

5.1 Occurrence of cardiac diseases in dogs

In the present study congestive heart failure **(**CHF) was diagnosed by utilising the history, physical examination findings, electrocardiography, radiography and

echocardiography. Routine hematology and blood biochemical analyses were used to rule out the presence of other diseases rather than in diagnosing CHF. Along with the imaging techniques, blood tests were used to differentiate CHF from other diseases causing similar clinical signs like hepatic disease, renal failure, pyometra, anemia, primary pulmonary conditions and pericardial effusion as depicted in Table 1 and Fig.1. The diagnosis of heart failure, especially when relying solely on symptoms and signs (which is often the case in primary care), is fraught with difficulties. In the current study, many of the symptoms of CHF, like ascites, exercise intolerance, dyspnoea, tachypnoea and muffled heart sounds were also seen in other diseases which can be confused for cardiac involvement even in the absence of heart ailments. Similar observations were made by Mosterd and Hoes (2007) who reported that many patients deemed to have heart failure will simply be found to be obese, have musculoskeletal problems, metabolic disorders, or pulmonary disease upon further examination.

It can be deduced from Table 2 and Fig. 2 that the occurrence of CHF in dogs in the current study was 0.45 % for the year 2014. The occurrence of MVD was 0.11 % and DCM 0.34% in dogs presented to the Veterinary College Hospital. Of the 78 dogs with CHF, 76 % (59) suffered from DCM and 24 % (19) MVD.

In a study conducted in Veterinary College Hospital, Bangalore in 2005, the occurrence was 1.17 % (Deepti, 2005). The diagnosis was based on clinical signs, electrocardiography and radiography. The low per cent noticed in the current study could be due to the use of echocardiography for the confirmation of cardiac disease as compared to diagnosis made earlier based on clinical signs and ECG referable to heart failure.

Earlier workers have reported the prevalence of MVD in dogs to be between 8% and 42% and DCM between 0.45% and l.l% (Woodfield *et al*., 1995b); DCM prevalence as 0.5% in Purdue University database in 1995, 1.1 % in an Italian study in 1988 and 1.5% at post-mortem in 1965 (Tidholm *et al*., 2001); atrioventricular valve endocardiosis 49.4% and myocardial diseases, predominantly dilated cardiomyopathy 21.1% in a study in Zurich (Baumgartner and Glaus, 2004); 8% of overall mortality in dogs < 10 years of age in a study of insured dogs with 0.75 % as the prevalence of heart disease of which 452 (15%) had MVD and 653 (21%) had cardiomyopathy (Egenvall *et al*., 2006), 4.83% in a study in the United States of America (Slupe *et al*., 2008) and 0.36% prevalence of MVD in a 2 year study in England (Mattin *et al*., 2015).

The variation in occurrence of CHF could be because of estimates based on high-risk breeds (higher prevalence), the criteria used for establishing diagnosis (antemortem v/s post-mortem), the breed and age distribution of the study population, geographical location and the practice setting (primary v/s referral) as indicated by Woodfield *et al*., (1995a), Egenvall *et al*., (2006) and Borgarelli and Buchanan (2012).

The breed-wise distribution of heart disease and CHF in the current study as depicted in Table 3 and Fig. 3 indicate that CHF was highest in the Labrador Retriever (32.05%), followed by Non-descript dogs (21.80%), Pomeranian (10.25%), Golden Retriever (8.98%), German Shepherd (5.12%), Mastiff, Doberman Pincher, Saint Bernard, Dachshund (3.85%), Boxer (2.56%) and Cocker Spaniel, Toy Poodle and Rottweiler (1.28 %) of which all the Pomeranian, the Cocker Spaniel, Toy Poodle and 6 ND dogs were diagnosed as MVD and the rest DCM.

The prevalence of MVD in small breeds of dogs as observed in the present study is similar to the findings reported by earlier workers who indicated that MVD was the most common heart disease in small-breed dogs even though other large breeds may be affected and the breeds were Cavalier King Charles Spaniel, Dachshund, Pomeranian, Yorkshire Terrier, Chihuahua and Poodle (Serfass *et al*., 2006; Borgarelli and Buchanan, 2012; Garncarz *et al*., 2013; Mattin *et al*., 2015). Based on the fact that some dog breeds are predisposed to the development of heart disease/ failure, it has been suggested that the disease has a strong genetic background as stated by Madsen *et al*. (2011).

Highest occurrence of DCM was found in the Labrador, followed by ND dogs and Golden Retriever in the current study which is different from the findings of earlier workers who reported purebred, large and giant dogs like the Doberman Pinscher, Boxer, English Cocker Spaniel, Portuguese Water Dog, Airedale Terrier, Newfoundland, St. Bernard, Standard Poodle, Scottish Deerhound, Irish Wolfhound and Great Dane as over-represented and commonly affected with DCM (Sisson and Thomas, 1995; Tidholm and Jonsson,1997; Dambach *et al*., 1999; Egenvall *et al*., 2006; Martin *et al*., 2009).

The reason Labrador Retriever, though not referred to in literature as a breed predisposed for developing DCM but over-represented in this study, could be that they are a very popular breed in Bangalore. Johnston *et al*., (2013) concluded in their study that this breed because of its popularity as family pets, may be functioning as sentinels for trends in general pet dog population. Other highly predisposed breeds have been under-

represented in the present study (like Doberman, Boxer, Great Dane), probably reflecting the demographics of Bangalore pet dog population.

In the current study, DCM was the most common cause of CHF. Similar findings were reported by Rajkumar (2013) in a study conducted at Veterinary College Hospital, Bangalore. Even though literature indicates MVD to be the most common disease of the heart in dogs (Ettinger and Suter, 1970; Linklater *et al*., 2007; Borgarelli *et al*., 2008; Olsen *et al*., 2010; Wolf *et al*., 2012), the opposite trend found in this study could be explained by the breed prevalence in the cases presented to this hospital. Labrador and Golden Retriever being medium- large breed dogs are more predisposed to developing DCM rather than MVD and as popular pets are over-represented in this study.

The age-wise occurrence of CHF as presented in Table 4 and Fig. 4 reveal that occurrence was highest in the dogs of 5-10 year old age group (42, 54 %) followed by < 5 years age group (23, 29 %) and >10 years age group (13, 17 %). The age of dogs with MVD ranged from 7 to 14 years with the mean age of occurrence being 10.39 ± 0.51 years and for DCM 1.5 to 12.0 years and 6.26 ± 0.31 years respectively. There was a significant difference in the means of age of the two groups ($P< 0.05$) indicating early onset of CHF for DCM dogs as compared to MVD dogs. Similar findings have been reported by earlier workers Rush (2002) and Haggstrom *et al*., (2009) who have observed that MVD is characterised by chronic progression with the condition worsening over several years and many dogs may not even show clinical signs of disease and may die of old age or other co-morbid conditions and the average age of dogs with CHF due to MVD was between 11 to 12 years (Garncarz *et al*., 2013). Earlier workers have reported the age of onset of CHF

with DCM as 4-8 years (Sisson *et al*., 2000), 6.6 years (Sleeper *et al*., 2002), 5 years (Borgarelli *et al*., 2006), 7.5 years (Meurs *et al*., 2007) and 8 years (Wiersma *et al*., 2008), which is a generally younger population of dogs than those afflicted with degenerative valvular disease. Perusal of literature did not indicate the reason why DCM occurs at a younger age than MVD.

In the present study, the gender-wise occurrence of CHF as depicted in Table 5 and Fig. 5 reveals that the condition was more common in males than females by more than 2 times for both DCM and MVD and the difference was statistically significant at $P < 0.05$. Similar findings have been reported by earlier workers who have observed that MVD is more common in male dogs than in female dogs (Rush, 2002, Serfass *et al*., 2006, Atkins *et al*., 2009; Garncarz *et al*., 2013) with progression of the disease faster in males than in females. A male predominance has been reported in for DCM by Tidholm *et al*., 2001 and Borgarelli *et al*., 2001. Cardiac related mortality in males and females was 27.3 deaths and 15.4 deaths per 10,000 dog years at risk (approximately twice), respectively in a study by Evengall *et al*. (2006). The reason for this pattern is not known. A possible reason could be that DCM may be an X linked recessive trait as in Great Danes as indicated by Meurs *et al*., 2001, however in another study on the epidemiology and mode of inheritance in four families of Dachshunds, X-linked dominant and X-linked recessive mode of inheritance were excluded as stated by Garncarz *et al*., 2013 indicating that X linked inheritance cannot be considered the cause for male predominance in all breeds of dogs.

5.2 Clinical signs in congestive heart failure

The frequency of occurrence of various clinical signs observed in the present study is presented in Table 6 and Fig. 6. Cough was the most common sign of CHF in this study (23.4%) and similar findings have been reported by earlier workers (Tidholm *et al*., 2001; Ristic, 2004; Smith 2006). Coughing is an important physiological function present in many mammalian species to remove or expel harmful substances, such as foreign bodies, mucus, or debris, from the airways and preserve the normal health of the respiratory tract. Cough can be evoked by stimulation of coughing receptors localized in the larynx, trachea, or bronchi, whereas irritation of smaller bronchi, bronchioles, and alveoli does not elicit coughing as indicated by Ferasin *et al*., (2013). Hence pulmonary edema should not be an expected cause of cough, unless fluid accumulation is severe enough to build up within the airways, producing a soft moist cough accompanied by blood-tinged sputum. Less commonly, disease processes involving the pleura, pericardium, diaphragm, nose, nasal sinuses, and mediastinum may also stimulate the coughing reflex as reported by Anderson-Wessberg (2010). Bigger than normal hearts in CHF can exert a mechanical dorsal pressure on the airways with subsequent stimulation of coughing receptors as indicated by Sisson (2010).

In this study ascites occurred in 25 dogs (18.3%) accompanied by peripheral edema in 4 dogs (3%) with DCM. Similar findings were observed by Sisson (2010) who reported that fluid accumulation occurs due to systemic venous congestion secondary to right sided heart failure. Peripheral edema may occur in cases of CHF with ascites, but it is unlikely that peripheral edema occurs without the animal first developing ascites in CHF (Ristic, 2004; Chambers, 2010). In the current study, ascites and peripheral edema occurred in

dogs with DCM which is similar to the findings by Mallery *et al*., (1999) who have also reported that ascites in isolated MVD is uncommon, but progressed MVD often tends to involve the right side of the heart as a consequence of pulmonary hypertension or development of a tachyarrhythmia. They also indicated that recurrent ascites is a common reason for euthanasia in dogs with CHF.

Anorexia occurred in 18 dogs (13.2 % of clinical signs) and may be secondary to fatigue or dyspnea. Anorexia may also occur in the treated dog due to medication toxicity (digoxin) or feeding of an unpalatable diet (sodium restriction) as indicated by Mallery *et al*., (1999). Freeman and Rush (2010) reported that increased production of inflammatory cytokines, such as tumor necrosis factor (TNF) and interleukin-1 (IL-1) in CHF can also directly cause anorexia. Ristic (2004) has reported that edema of the gut also causes anorexia.

Dyspnoea occurred in 18 dogs (13.2% of clinical signs) and tachypnoea in 6 dogs (4.4%) in the current study and these dogs had radiographic evidence of pulmonary edema or pleural effusion. This is similar to the observations of Forney (2010), Fonfara *et al*. (2011) and Ferasin *et al*. (2013) who reported that pulmonary edema is inevitably presented with dyspnea or tachypnea.

Exercise intolerance (8 dogs, 5.9 %) and lethargy (4 dogs, 3%) were observed in some dogs in the present study. The causes could be due to reduced cardiac output leading to diminished oxygen delivery to the tissues, arrhythmias or by cardiac cachexia as indicated by Freeman (2012). In the present study, other than sinus tachycardia (which

may be physiological or pathological), 20 dogs had arrhythmias of which atrial fibrillation could be attributed as the cause of exercise intolerance in 5 dogs and ventricular tachycardia in one dog. However, unless the dysfunction is of sufficient severity to induce exercise intolerance, the clinician must continue evaluation for concurrent conditions that may be the actual cause of weakness because exercise intolerance or lethargy in an older dog may be caused by concurrent conditions like endocrine disorders, renal or hepatic insufficiencies, anemia and neoplasia as indicated by Schulman (2010). Thus in the present study, routine hematology and biochemical analysis was carried out in every case suspected for suffering from heart failure.

Epistaxis/ hemoptysis occurred in 7 dogs (5.1%). Most dogs in the present study with hemoptysis/ epistaxis had occasional blood mixed small quantity of fluid being brought out with two exceptions. One dog had watery discharge not mixed with blood even though it has been classified under epistaxis/ hemoptysis. The animal was bringing out significant amounts of such fluid from the mouth even without coughing. Applying pressure on the thorax or abdomen to put him on the table or on the ground was sufficient to cause the fluid discharge. One dog had viscous pinkish red fluid resembling “water melon juice” which the owner initially thought was hematemesis. Epistaxis or hemoptysis occurs in severe cases of cardiogenic pulmonary edema where a moist cough is accompanied by blood tinged frothy fluid as reported by Ferasin *et al*., 2013. Detection and confirmation of hemoptysis may be difficult in dogs because they do not expectorate after coughing as stated by Geiger, 2010. To direct the diagnostic and therapeutic process, it must be determined that the coughed up material is actually blood and that the blood was coughed up and not regurgitated or vomited because treatment of perceived hematemesis

with fluid therapy will have disastrous consequences in hemoptysis due to cardiogenic pulmonary edema. In the present study, a simple pH test was done on the coughed up fluid to ensure that it was pulmonary edema fluid and not the acidic gastric contents.

Three dogs had wheezing/ noisy respiration (2.1% of clinical signs) in the present study. These signs were reported by the owner rather than being observed at the hospital. One dog with this symptom had elevated trachea as seen on the radiograph. This corroborates with the observations of Ettinger (2010) who indicated that wheezing is a clinical sign of tracheo-bronchial disease and may occur due to narrowed trachea or bronchi.

Orthopnoea was observed in 3 dogs (2.1%) in the present study. These cases were accompanied by epistaxis/ hemoptysis. Orthopnoea is difficulty in breathing unless in an upright position (Forney, 2010) and occurs due to hypostastic congestion of lungs and pulmonary edema (Ristic, 2004).

Syncope was also observed in 3 dogs (2.1%) and cyanosis and pallor in 2 dogs each (1.4%). In the current study, syncope was associated with atrial fibrillation, ascites, and epistaxis in one dog and tachycardia in the other two. Two dogs with cyanosis had tachycardia but two dogs with pallor had unremarkable ECG and haematological findings. Similar findings were reported by Olsen *et al*., (2010) who indicated that tachyarrhythmias may be accompanied by syncope and that syncope and/or signs of forward failure (cyanosis, pallor) may also be present in CHF. Rush (2002) has observed that coughing which is a common sign of CHF can also cause syncope (tussive syncope). Smith (2006) is

of the opinion that pallor or the paleness of a tissue is caused by decreased tissue perfusion ("shock") which can be confused for anemia. Presence of intermittent cyanosis with pallor or syncope rules out anemia as does a blood test (Ortega-Simpson, 2010). Cyanosis occurs most commonly due to cardiovascular, pulmonary (edema), or other diseases resulting in ventilation-perfusion abnormalities (Allen, 2010).

Weight loss occurred in one dog (Doberman with DCM). Freeman (2012) has observed weight loss more commonly in DCM as seen in the current study as well. Weight loss can occur due to anorexia seen in CHF, but may also be due to cachexia (loss of lean body mass or muscle wasting) as stated by Dove (2001).

Diarrhoea occurred in one dog (0.7%). Diarrhoea occurs due to edema of the intestines caused by systemic venous congestion as indicated by Ristic (2004).

The auscultable abnormalities in the current study (Table 7 and Fig. 7) were tachycardia in 35 (44.87%), murmurs in 16 (20.51%), gallop rhythm in 5 (6.41%) and muffled heart sounds in 5 dogs (6.41%).

Murmurs in the current study were detected in 14 cases of MVD and 2 cases of DCM. This observation is similar to the results of earlier workers who reported that heart murmurs are very common in CHF due to MVD (Smith, 2006) and that a typical heart murmur for chronic valve disease was noted in most dogs with a positive diagnosis (Garncarz *et al*., 2013). Though heart murmurs are common, they may not be heard in all cases of MVD as observed by Mattin *et al*., 2015 and murmurs may also be detected in some dogs with DCM as reported by Monnet *et al*., 1995 and Tidholm *et al*., 2001.

In the current study, all dogs with gallop rhythm had DCM and the gallop rhythm occurs due to the presence of extra heart sounds and is not truly a rhythm and when present, indicates diastolic volume overloading as in dilated cardiomyopathy and mitral insufficiency as observed by Prosek (2010).

In the present study, 5 out of 8 dogs with pleural effusion had muffled heart sounds. This is in agreement with the statement of Ludgiw *et al*., 2010 that muffled heart sounds can occur due to pericardial/ pleural effusion. However benign cause of such sounds can be obesity as observed by Barrett (2010).

Crackles were observed in 7 dogs indicative of fluid build up in the lung and in the present study, 42 dogs had pulmonary edema. The reason for only a small proportion of dogs having crackles could be that crackles are most common in pulmonary fibrosis than in pulmonary edema as indicated by Hughes (2012). Also presence of pleural fluid could have masked lung sounds in some of the cases (8 dogs).

It is a well known fact that auscultation is one of the true arts of veterinary medicine but it can be learnt and perfected with some diligence and perseverance. It requires a methodical approach and a good stethoscope. The animal in the standing or sitting position in a primary requirement for clear audibility and a good technique involves auscultation of both sides of the thorax. All cases of CHF did not have an auscultable abnormality in the present study and similar observations have been made by Borgarelli and Buchanan, 2012; Hughes, 2012 and Garncarz *et al*., 2013, however its presence is useful not only in detecting the existence of a problem but also diagnosing it in many

situations, for example, pulmonary crackles and cardiac murmurs may indicate pulmonary edema due to heart disease. Also in many situations like primary care facilities, this may be the only tool available for diagnosis of thoracic problems. Hence, cardiac auscultation is an important tool in the clinician's armamentarium.

It is evident from Table 8 and Fig. 8 that in the present study, 63 dogs (80.77%) belonged to "C2" group heart failure class of which 18 had MVD and 45 had DCM. Dogs with severe to life-threatening HF were classified as "C3" and 13 dogs (16.67%) belonged to this group of which one had MVD and the rest 12 DCM. Two dogs (2.56%) with refractory heart failure were classified as "D" and these had DCM.

The purpose of CHF cases being classified is that the class an animal belongs to, serves as clinical risk prediction tool that is helpful in guiding medical decision making. Patients estimated to be at a lower risk may be managed with less intensive monitoring and therapies, whereas a patient estimated to be at a higher risk may require more intensive management as stated by Fonarow *et al*. (2005). Classification has traditionally been used to grade severity of heart failure and indicate prognosis and, thus, to guide patient management. This system is essentially a functional/symptomatic score, not taking into account the underlying cardiac disorder that will almost inevitably progress, with the animal's improvement or deterioration changing the class it is allotted to as observed by Mosterd and Hoes (2007). These are subjective measurements made by the clinician with inputs from the patient owner to grade the severity of functional limitation and will depend on the clinician's interpretation of the animal's condition and are frequently used in research or clinical trials as an inclusion/ exclusion criterion and also as a measure of

outcome as reported by Raphael *et al*., 2007. Guidelines for the management of MVD in dogs was recently introduced as the American College of Veterinary Internal Medicine consensus statement (Atkins *et al*., 2009) where treatment and prognosis are based on heart failure class.

5.3 Electrocardiographic findings in congestive heart failure

In the current study, ECG was mostly performed in right lateral recumbency, but in orthopnoeic and dyspnoeic dogs, standing or sternal recumbency position was used even though varying the position can lead to some alteration in the morphology of the complexes recorded. However this was not clinically important and the rhythm, which is the most important aspect of the recording, will remain unaffected as indicated by Johnson (2008).

From the Table 9 and Fig. 9, it is evident that arrhythmias were seen in 49 cases. The auscultable tachycardia was further classified as sinus, atrial or ventricular tachycardia by electrocardiography as indicated by Oyama (2009) and Rasmussen *et al*. (2012).

In the current study, sinus tachycardia (Fig. 11) was the most common abnormality and was recorded in 29 dogs (59.18 % of arrhythmias). It is a sinus rhythm that occurs at an elevated rate marked by sympathetic predominance over parasympathetic inputs and is almost invariably a result of, rather than a cause of, a patient's problems according to Cote (2010). Sinus tachycardia may be physiological or pathological and its mere presence, even though should raise suspicion, will not confirm cardiac problems.

Atrial fibrillation (AF) was seen in 14 dogs (28.57 %) and was recognised by the disorganized atrial electrical activity resulting in an absence of P waves and a rapid, irregular ventricular rate (Fig. 12, 13). Two dogs which had sinus tachycardia at the first visit developed atrial fibrillation by the second visit indicating progression of the disease. In the present study, AF was the second most common abnormality and seen in dogs with DCM which is in agreement with the findings of earlier workers (Moise, 1999; Tidholm *et al*., 2001; Sleeper *et al*., 2002). Saunders *et al*. (2009) have reported that the hemodynamic consequences of AF include decreased cardiac output and the development of clinical signs of heart failure. Hence in the present study, dogs with AF were treated with digoxin for the management of AF when diuretics and ACE inhibitors failed to revert the abnormal rhythm to sinus rhythm or control the rate of AF.

Ventricular premature complexes (VPC) (Fig. 14) were observed in only 2 dogs with DCM in the current study even though these arrhythmias are the most common of all pathologic rhythm disturbances in dogs. Causes of ventricular extrasystoles include virtually any cardiac or systemic disorder, with the most common of these including such primary cardiac diseases as cardiomyopathy and valvular heart disease as stated by Cote (2010). Since isolated VPC do not cause hemodynamic abnormalities (Tilley, 1992), no specific treatment was attempted for controlling this arrhythmia.

Atrial flutter (Fig. 15) was recognised in one dog as a rapid and regular series of atrial depolarizations and occurs due to a micro-reentry pathway as stated by Cote (2010).

Ventricular tachycardia (VT) (Fig. 16) was seen in one Labrador Retriever even though VTs are very common in Dobermans as stated by Moise, 1999 and Tidholm *et al*., 2001. The reason for this could be that in the present study, Labradors are over-represented. No specific antiarrhythmic drugs were used in this case as VT resolved with treatment for CHF.

Right bundle branch block (RBBB) was seen in one dog. RBBB (Fig. 17) was differentiated from VT by the presence of a P wave preceeding every QRS as described by Cote (2010) where as in VTs, P wave if present can be observed before, during or after a QRS complex.

Premature atrial contraction (Fig. 18) was seen in one dog and was characterised by P on T phenomenon as described by Tilley, 1992.

In the present study as indicated in Table 9 and Fig. 10, morphological abnormalities were seen in 17 cases and tall R waves, i.e. more than 2.5-3.0 mV depending on the breed (7 dogs, 41.2%) was the most common finding. Increased height of R (Fig. 13, 20) waves may indicate left ventricular enlargement (Tilley, 1992; Cote, 2010), but ECG is not a very sensitive indicator of myocardial mass as in the present study, 51 cases had cardiomegaly by thoracic radiography and only7 cases had tall R waves in ECG recording.

Tall T waves (Fig. 19), i.e. more than one fourth the R wave were seen in 5 dogs. Myocardial disease or electrolyte abnormalities may cause T wave changes as described by Tilley, 1992. In those 5 cases, serum sodium and potassium levels were within normal range, indicating T wave changes to be due to myocardial involvement.

Deep Q in Fig. 20 (> 0.5 mV in Lead II) and deep S in Fig. 21 (in Leads I, II, III and aVF) observed in the ECG of 2 dogs were suggestive of right ventricular enlargement or hypertrophy as described by Tilley, 1992 and these findings correlated clinically by the presence of ascites in such dogs.

Short R waves, i.e. less than 0.5 mV was observed in 2 dogs. Consistently low-amplitude R waves suggest pericardial or pleural effusion or obesity as indicated by Tilley (1992) and Cote (2010). In the present study, one dog had pleural effusion and small R waves and the other did not, suggesting that obesity was the cause of the change in this dog's ECG and that this was a benign finding in the dog.

ST segment sagging (Fig. 22) was seen in 1 dog. The ST segment depression may be associated with myocardial hypoxia, nonspecific electrolyte changes, or cardiac hypertrophy as stated by Cote, 2010, but in this dog, non-specific electrolyte changes were ruled out as serum sodium and potassium remained within normal levels (Sodium 145 mg/dL and potassium 4 mg/dL).

However, the ECG abnormalities found by the earlier workers in DCM included atrial fibrillation, ventricular ectopy and ventricular tachycardia (Monnet *et al*., 1995); atrial fibrillation in 30% of the dogs (Calvert *et al*., 1997); atrial fibrillation (most commonly diagnosed electrocardiographic abnormality), ventricular premature depolarizations and ventricular tachycardia (in majority of Doberman Pinschers) (Tidholm *et al*., 2001) and isolated supraventricular ectopic beats, isolated VPC, ventricular tachycardia and atrial fibrillation (Borgarelli *et al*., 2006). In MVD, left atrial enlargement

(P mitrale; P > 0.04 sec), and infrequently P pulmonale (P > 0.4 mv), ST segment slurring and ST depression, sinus tachycardia, supraventricular arrhythmias and atrial fibrillation were observed and ventricular arrhythmias were found to be uncommon (Rush, 2002; Smith, 2006; Garncarz *et al*., 2013). The latter two arrhythmias were more common in decompensated or serious conditions of MVD.

Even though the ECG is an invaluable tool in the diagnosis of cardiac problems (when arrhythmias are present), it has many limitations. The ECG is not usually an accurate guide to heart size. If cardiomegaly is suspected, other tests particularly radiology should be considered as indicated by Schober *et al*., 2010. A dog with CHF may have a normal ECG, as seen in this study (14 dogs) and a perfectly normal animal may show nonspecific ECG abnormalities. Therefore one should avoid reading too much into borderline changes and serial tracings over a period of time are of greater value in evaluating the functional status of heart as suggested by Tilley, 1992; Johnson, 2008 and Cote, 2010.

5.4 Radiographic findings in dogs with congestive heart failure

The radiographic findings in CHF cases are presented in Table 10 and Fig. 23 to 28. The findings were cardiomegaly in 51 (86.44%), elevated trachea in 39 (66.1%), pulmonary edema/ congestion (hypervascularisation of lungs) in 42 (71.18%), reduced cardiophrenic angle in 39 (66.1%) and pleural effusion with obscure cardiac silhouette in 8 (13.56%) and ascites in 3 cases (5.1%). Similar findings have been reported by earlier workers (Woodfield *et al*., 1995a; Tidholm *et al*., 2001; Rush, 2002; Martin *et al*., 2009) who have very commonly observed pulmonary edema, cardiomegaly, straightening of the caudal cardiac border and loss of the caudal cardiac waist, elevation of trachea and

compression of the left mainstem bronchus, pleural effusion, and abdominal distention caused by ascites.

Thoracic radiography can be employed to provide further evidence of the disease when cardiac involvement is strongly suspected. Contrary to the common opinion that radiography is only suitable for structures like bones, radiography using a good technique gives a great deal of information about soft tissues like the heart and lung. Advances like computed and digital radiography have drastically improved the image quality. However, it cannot be used to conclusively differentiate between various heart diseases. In the current study, it was invaluable in differentiating cardiac from respiratory disease (2 cases of pulmonary neoplasm and one case of pneumothorax), and to determine whether CHF is present. Thoracic radiography is the most commonly applied method for the diagnosis of CHF and is considered the clinical ‘‘gold standard’’ and the only test for heart conditions which can also assess the pulmonary condition as indicated by Smith, 2006 and Schober *et al*., 2010.

However, radiography is of unspecified sensitivity (milder cases may be missed) and specificity (pericardial effusion from cardiomegaly), especially in the setting of combined heart and lung disease (in many small breed dogs), and can suffer from considerable observer variation as stated by Schober *et al*., 2010 and Ferasin *et al*., 2013. Hence to overcome the errors associated with subjective evaluation, vertebral heart score (VHS) was suggested by Buchanan and Bucheler (1995) as an objective measurement of cardiac size to diagnose cardiomegaly, but recent studies have found that inter-observer variability can also occur with VHS scores and many normal animals may have higher than normal scores (Hansson *et al*., 2005; Jepsen-Grant *et al*., 2013).

5.5 Hematology and routine biochemical test results in congestive heart failure

It is evident from Table 12 that the haematological and routine biochemical parameters were within normal range except for a few outliers.

Seven dogs had mild leucocytosis (> 17000 – 25000/µL) and 4 had moderate leucocytosis (> 25000/µL). The levels in subsequent visits were in the normal range even though they were treated for CHF without any antimicrobial agents in the regimen. Twenty one animals had mild to moderate anemia (haemoglobin values ranged from 7.9 g/dL to 11.6 g/dL). The dog with the least haemoglobin in the study group (7.9 g/dL) also had wounds which may explain the decrease in haemoglobin content. Three dogs had platelet count of less than one lakh and in subsequent visits, the level had come back to normal. In the present study, four dogs with moderate leukocytosis had severe to life threatening CHF and were classified as C3. Similar to the findings in the present study, Farabaugh *et al*. (2004) have reported that leukocytes were significantly higher in the CHF group as compared to the controls, and hemoglobin significantly lower. Their study also indicated that the leukocytes increased and the hemoglobin decreased with increase in the heart failure class. They also reported that the clinical implications of these findings are not known, but in human beings, low hemoglobin levels are predictors of mortality. The probable reason for changes in the hematology parameters could be related to enhanced corticosteroid production or other neurohormonal alterations that occur in heart failure and many cases of CHF may present with a stress leukogram and an elevated leukocyte count as stated by Ristic, 2004. Farabaugh *et al*., 2004 also reported significantly elevated platelet counts in CHF dogs which is dissimilar to the findings of the current study. They also indicated that the clinical significance of these changes is unknown and perusal of literature did not shed any light on this issue.

In the present study, four of the dogs had creatinine values more than 1.4 mg/dL. Earlier workers Boswood and Murphy, 2006 and Olsen *et al*., 2010 have also encountered increased creatinine which indicates prerenal azotemia or more advanced CHF. Twenty three dogs had mild elevated ALT values. Similar findings were reported by Ristic, 2004 and Olsen *et al*., 2010 who indicate that such an increase is probably due to hepatic congestion. In the current study, mild variation was seen in both the potassium and sodium values as depicted in Table 12 but no specific ECG abnormalities could be attributed to them. However hypokalemia and hyperkalemia both have adverse effect on the heart and may produce ECG abnormalities and potassium and sodium may decrease in conditions of advanced heart failure as indicated by Boswood and Murphy, 2006.

In the current study, hematology and biochemistry were not very useful for the diagnosis of heart disease though they have been helpful in investigating potential concurrent disease. This is similar to the findings of earlier workers (de Morais, 2000; Dukes-McEwan *et al*., 2003; Boswood and Murphy, 2006; Olsen *et al*., 2010) who have stated that hematology and biochemistry were more useful in ruling out other diseases. It is important that the clinician should not over interpret small changes from reference intervals as only slightly more than one third of normal animals are likely to have normal results in all tests of a 20 test profile as stated by Willard and Tvedten, 2012.

5.6 Cardiac biomarkers in congestive heart failure

A total of 36 serum samples of dogs with CHF and 10 normal dogs were used for testing the biomarker levels and to compare these results with the conventional tests (echocardiography). Currently rapid assay or bed-side kits are not available for cardiac

troponin I (cTnI) for dogs in our country and no rapid bed-side kit is available for canine amino terminal pro B-type natriuretic peptide (NT-proBNP) anywhere in the world, hence canine specific sandwich based ELISA kits were used in the present study.

Mean creatine kinase, myocardial band (CK-MB) in serum samples of CHF and normal dogs were 15.86 ± 0.66 U/L and 12.3 ± 1.1 U/L respectively. There was a considerable overlap of values between the groups and only 12 dogs with CHF had values more than that of the normal dogs and therefore CK-MB is not specific to heart disease which is similar to the findings by Schober *et al*., 1999 and Dolci and Panteghini, 2006.

In the present study, cTnI was measured by canine specific sandwich ELISA technique (Fig. 29) with a detection range of 0.156-10 ng/ dL and a qualitative human immunochromatography test kit (Fig. 30 a to d) with an analytical sensitivity of 0.5 mg/mL. The human kit was validated for canine cTnI using the cTnI standard from the canine cTnI sandwich ELISA kit and was able to detect up to 0.156 ng/ mL. ELISA technique was able to quantitate cTnI in 7 cases and the rest were assumed to be zero (undetectable, hence zero). The detected values ranged from 0.4 ng/ mL (found in a normal dog) to > 10 ng/mL. The value of cTnI in normal animals according to earlier studies were 0.01-0.08 ng/mL (Oyama and Solter, 2004; Linde *et al*., 2006; Mellor *et al*., 2006; LaVecchio *et al*., 2009) and in cardiac disease 0.03- 5.47 ng/ mL (Oyama and Solter, 2004; Prosek *et al*., 2007). The kit used in the present study was unable to quantify the value of cTnI in normal dogs except one, but since the mean values in CHF dogs in literature was more than the analytical sensitivity of the kit, it was decided to utilize this canine specific assay kit. In most of the literature, very sensitive human ELISA kits have been used after validating them, but since those were unavailable to us, the less sensitive, canine specific kit was used. It is possible that because of the decreased analytical sensitivity of the kit,

some dogs with myocardial damage and cTnI levels between 0.03 to 0.156 ng/mL were missed.

Contrary to the ELISA kit, the human immunochromatographic test with 0.5 ng/mL analytical sensitivity was able to detect the presence of increased cTnI values in 19 of the serum samples from CHF dogs as compared to the 6 in the ELISA kit. This indicates that the human cTnI immunochromatography kit (detected 19 samples out of 36) was more sensitive than the canine specific cTnI ELISA kit (detected 6 out of 36).

NT-proBNP was also analysed using a sandwich ELISA kit (Fig. 31) with the detection range of 0.312 to 20 ng/mL. Since the unit “pmol/L” has been quoted in literature, the conversion was done using the formula “ NT-proBNP in pmol/L is equal to NT-ProBNP in pg/ml divided by 10.545” given by Hytest company (2013). In the CHF group, the values ranged from undetectable to 1449 pmol/L and in the normal group undetectable to 58 pmol/L.

The values in normal and CHF dogs for NT-proBNP is being revised from time to time due to the increase in the sensitivity of the ELISA tests by improvement being made with the capture and detection antibodies utilised in the tests and by better sample handling, as indicated by Collins, 2013. Earlier studies indicate a wide range of values in health (80.2-831.97pmol/L) and disease (> 1200 up to 6500 pmol/L) in dogs (Reynolds and Oyama, 2008; Schober *et al*., 2010; Ebisawa *et al*., 2012; Wolf *et al*., 2012).

Two samples had both high cTnI and NT-proBNP, 2.072 ng/mL and 58 pmol/L in one dog and > 10 ng/mL and 605 pmol/L in another respectively. High cTnI and NT-proBNP values together could diagnose 10 cases of CHF. cTnI and NT-proBNP were not elevated in the same samples in all cases. This could be due to different mechanism of

release of these biomarkers into the serum, cTnI due to myocardial injury as reported by Braunwald, 2008 and Boswood, 2009 and NT-proBNP due to myocardial stress or stretch as stated by Kimmenade and Januzzi, 2009 and Wolf *et al*., 2012.

5.7 Echocardiography in dogs with congestive heart failure due to DCM and MVD

Echocardiography was required to confirm the diagnosis of CHF by identifying the characteristic features of the disease as indicated by Smith, 2006. Myocardial hypokinesis measured as low fractional shortening (FS) and left atrial and ventricular dilatation without other detectable cardiac abnormalities have been regarded as diagnostic criteria for DCM and demonstrating mitral regurgitation and enlarged left atrium and ventricle for MVD and this can be confirmed by echocardiography as reported by Tidholm *et al*., 2001; Dukes Mc-Ewan *et al*., 2003; Chetboul and Tissier, 2012 and Garncarz *et al*., 2013. In the present study, based on echocardiographic criteria 59 cases were diagnosed as DCM and 19 cases as MVD (Tables 13 and 14).

Left ventricular internal diameter at diastole ($LVID_d$) and left ventricular internal diameter at systole ($LVID_s$) were used to measure FS (Fig. 39b) which is the per cent change in diameter of the ventricular cavity from diastole to systole and it provides a rough index of cardiac function. The FS is the clinical index used most commonly in the evaluation of global inotropism and systolic function in veterinary medicine according to Dukes-McEwan *et al*., 2003 and Belanger, 2010 who give the range in normal dogs as 27-48%. FS is a major criterion in the diagnosis of DCM as indicated by Dukes-McEwan *et al*., 2003 and Petric and Tomsic, 2008. In the present study, FS in DCM dogs ranged from 6.56 to 22.86% and mean ± S. E. was 14.06 ± 0.55 % which is well below the normal values, confirming the diagnosis of global left ventricular hypokinesis and DCM.

Ejection fraction (EF) in normal dogs ranges from 50-65% and less than 40% is considered abnormal as reported by Dukes-McEwan *et al*., 2003. In the present study, ejection fraction ranged from 14.46 to 39.33 % with mean 26.51 ± 1.78 % in DCM dogs. Decreased EF is one of the major criteria for diagnosis of DCM along with decreased FS. EF is more commonly utilised for diagnosis of systolic dysfunction in humans, whereas FS is most commonly used in animals as reported by Dukes-McEwan *et al*., 2003and Petric and Tomsic, 2008.

In the present study, E-point to septal separation (EPSS) ranged from 0.79 to 1.51 cm with the mean and S. E. as 1.11 ± 0.02 cm in DCM dogs. Normal values in dogs range from 0.3 to 0.8 cm and increased EPSS is considered a minor criteria in the diagnosis of DCM as indicated by Dukes-McEwan *et al*., 2003. EPSS measures the distance from the maximum opening of the mitral valve (E point) to the endocardial aspect of the interventricular septum (Fig. 38) and is a useful parameter in the assessment of left ventricular dilation and systolic dysfunction. In dilated hearts, where there is decreased contractility (such as in dilated cardiomyopathy), the mitral valve does not reach the septum as reported by Belanger, 2010.

End systolic volume can be normalised to the body surface area and expressed as end systolic volume index (ESVI) in ml/m^2 (Dukes-McEwan *et al*., 2003; Petric and Tomsic, 2008). ESV-I <30 ml/m^2 is suggested as normal in dogs. In the present study, the ESVI ranged from 25.82 to 169 mL/m^2 and the mean ± S. E. was 56.52 ± 10.69 mL/m^2 in DCM dogs. Two dogs had ESVI <30ml/m^2, but their EF and FS were lower than normal, so they were still classified as DCM. Increased ESVI offers unequivocal evidence for systolic dysfunction as indicated by Dukes-McEwan *et al*., 2003.

In the MVD, the LA/Ao (Fig. 41) ranged from 1.71 to 2.32 with a mean 1.96 ± 0.04. This was more than the reference range (0.52-1.13) for normal dogs as indicated by Boon (2011) and Chetboul and Tissier (2012) who also indicated that a ratio > 1.7 definitely indicates left atrial enlargement. Mitral regurgitation was severe (Fig. 37) in 3 dogs of which one had epistaxis/ hemoptysis (grouped as C3) and moderate (Fig. 36) in the rest 16 dogs. In the present study, the severity of mitral regurgitation (MR) correlated with the severity of clinical signs which is similar to the observation made by Chetboul and Tissier (2012) that evaluation of MR severity is of critical importance in dogs with MVD, as MR directly reflects the primary hemodynamic consequence of incomplete apposition of the mitral valve leaflets during systole. The cause for the mitral regurgitation, which is the mitral valve degeneration (Fig. 35) was appreciated echocardiographically in 13 cases.

The range and mean of echocardiographic parameters in MVD for EPSS, ejection fraction and fractional shortening (Fig. 39a) were 0.28 to 0.71 cm and 0.44 ± 0.03 cm, 51.89 to 64.44 % and 59.11 ± 2.63 % and 31.11 to 56 % and 41.18 ± 1.60 % respectively and were within normal range or mildly elevated for FS and EF. Similar findings have been observed by Bonagura and Schober (2009) and Trafny *et al*., (2012).

Pleural effusion (Fig. 42) observed in 8 dogs by radiography and ascites (Fig. 43) in the current study could also be confirmed in the dogs suffering from CHF by ultrasonography. These clinical signs were seen in bilateral congestive heart failure and were due to DCM.

5.8 Prognostic potential of cardiac biomarkers

In the current study, elevated cTnI and NT-pro BNP values were associated with increased severity of clinical signs, presence of atrial fibrillation, elevation of creatinine

and DCM which is similar to the findings of Prosek *et al*., 2007; Ljungvall *et al*., 2014, Carreton *et al*., 2014 who have indicated that elevated cardiac biomarkers are associated with increased severity of the condition.

In the current study, high NT-proBNP values were associated with azotemia. Similar observations in both man and dog have been made by earlier workers (MacDonald *et al*., 2003; Weber and Hamm, 2006; Srisawasdi *et al*., 2010). Raffan *et al*., 2009 reported that NT-proBNP has no known receptors and little is known about its clearance from plasma, although renal metabolism is thought to be responsible. This explains the reason why high NT-proBNP values were high in CHF with concurrent azotemia. But perusal of literature did not explain the reason why cTnI were also very high in renal azotemia.

In the current study, high biomarker values were seen in DCM probably due to the reason that DCM was presented with more severe form of CHF than MVD dogs.

Though radiography and echocardiography are gold standards for diagnosis of CHF and cardiac diseases respectively, circulating cardiac biomarkers continue to generate lot of interest and research as indicated by Schober *et al*., 2010. The wide variation in chest conformation, phase of respiration, radiographic technique, and co-morbidities (eg, obesity, combined cardiac, and pulmonary disease) often make radiographic evaluation difficult as indicated by Bahr, 2013. Echocardiography is commonly used to document underlying cardiac dysfunction (valvular disease and myocardial disease) but its availability may be limited. Furthermore, dyspnoeic patients (a common sign of CHF) also may be too unstable to permit manual restraint required for radiographic or echocardiographic study, whereas, in others, the quality of the study may be adversely affected by obesity, patient position and movement, or pulmonary disease. Therefore, even

when radiography and echocardiography are available, an accurate, sensitive, and specific blood test for biomarker quantification for differentiating CHF from other causes would be a useful addition to the clinician's diagnostic armamentarium as opined by de Madron *et al*., 2011. Another advantage of a biomarker over imaging techniques would be the objectivity of the results obtained.

5.9 Treatment trial

In the present study, 24 animals were selected for therapeutic trials and randomly allocated to 4 treatment groups, viz., Group I (treated using conventional protocol i.e., frusemide, spironolactone, enalapril with or without digoxin), Group II (treated with conventional protocol along with nutraceutical), Group III (treated with conventional protocol along with pimobendan) and Group IV (treated with conventional protocol along with pimobendan and nutraceutical). The groups were allotted scores based on owners and clinicians subjective impressions regarding the resolution of or improvement of clinical signs and this is presented in Table 17 and Fig. 35. All dogs tolerated the medication well without untoward side effects. Group IV had the lowest mean score (0.22) despite a death in this group followed by Group III at 0.31, Group II at 0.55 and Group I least at 1.34. A dog died in Group I (1/6) and one in Group IV (1/6) during the monitoring period. The reason for death could be attributed to the severity of the clinical signs at the beginning of the trial rather than the protocol itself.

Pimobendan exerted a beneficial effect in the present study. The mean scores in the groups containing pimobendan (III, IV) were higher than those without (I, II). Owners reported increased activity levels in dogs of Group III and IV. Pimobendan is a phosphodiesterase III inhibitor and calcium channel sensitizer causing a inodilating effect,

at reduced myocardial oxygen consumption and reduced myocardial energy expenditure when compared to other positive inotropic agents (cardiac glycosides and catecholamines) as indicated by Fuentes, 2004 and Atkinson *et al*., 2009 which would explain the effect seen in the present study.

The nutraceutical also had an ameliorating effect on the clinical parameters as evidenced in Table 17. The lowest score was seen in the group administered pimobendan with nutraceutical along with conventional treatment. The owners also reported that the dogs had good to very good appetite as evidenced by increased food intake. The discovery of taurine and carnitine deficiency/ responsive dilated cardiomyopathies in the 1980s and 1990s fuelled hopes of reducing the prevalence of dilated cardiomyopathy in dogs by their supplementation in diet or in the form of medicine as reported by Gavaghan and Kittleson, 1997 and Keene, 2002. Ito and Azuma, 2012 have observed that unfortunately only small percentage of dogs respond to taurine and/ or carnitine supplementation completely indicating that the rest had idiopathic DCM. Anectodal evidence in humans and animals suggests that other nutraceuticals like coenzyme Q10, vitamin E, polyunsaturated fatty acids, selenium and betacarotene may be beneficial in the treatment of congestive heart failure as reported by Freeman *et al*., 1998; Dove (2001) and Keene (2002). In the current study, the serum levels of taurine, carnitine and other antioxidants were not quantified to diagnose DCM. However supplementation with nutraceuticals, even if it did not completely eliminate the need for use of conventional drugs, were found to be beneficial in the current study and without any adverse effects which is similar to the observations of Dove, 2001 who reported improvement in the quality of life or significant survival benefits of nutraceutical supplementation. One concern with the use of newer drugs like pimobendan and nutraceuticals is the cost of medication, which can be very high indeed

for large breed dogs, but given the improvement in quality of life, veterinarians and owners may be justified in prescribing and administering them respectively.

SUMMARY

VI SUMMARY

The present work was undertaken to study the occurrence of congestive heart failure due to dilated cardiomyopathy and mitral valve disease in the dogs presented to the Veterinary College Hospital during the year 2014, to study the utility of biomarkers cardiac troponin I and N-terminal pro B-type natriuretic peptide in heart diseases, to study the echocardiographic changes in dogs with DCM and MVD and to assess the potential of biomarkers and echocardiography in the diagnosis and prognostication of heart failure.

The occurrence of CHF in the cases (primary and referred) presented to Veterinary College Hospital in the year 2014 was 0.45% with DCM accounting for 0.34% and MVD 0.11%. This diagnosis was made based on history, thorough physical examination, routine blood tests (hematology and biochemistry), electrocardiography, radiography and echocardiography.

The highest occurrence of CHF was recorded in the Labrador Retriever (32.05%), followed by Non-descript dogs (21.80%), Pomeranian (10.25%), Golden Retriever (8.98%), German Shepherd (5.12%), Mastiff, Doberman Pincher, Saint Bernard, Dachshund (3.85% each), Boxer (2, 2.56%), Cocker Spaniel, Toy Poodle and Rottweiler (1.28 % each). Pomeranian, Non-descript dogs (6 dogs), Dachshund, Cocker Spaniel and Toy Poodle were the breeds affected with MVD and the rest from DCM. The reason for this occurrence could be due to breed popularity rather than breed predisposition to cardiac diseases. The occurrence was highest in the dogs of 5-10 years of age (42, 54 %) followed by < 5 years of age (23, 29 %) and more than 10 years of age (13, 17 %) with onset of CHF in DCM dogs being significantly higher at a younger age group than in MVD dogs. The occurrence of CHF in male dogs was 69.23 % (54 dogs) and 30.77 % in female dogs (24

dogs) and it was significantly higher in males as compared to female dogs in both DCM and MVD.

The clinical signs in CHF were coughing, ascites, anorexia , dyspnoea, exercise intolerance, epistaxis/ hemoptysis, tachypnoea, peripheral edema, lethargy, wheezing/ noisy respiration, orthopnoea, syncope, pallor, cyanosis, weight loss and diarrhoea. Auscultation of these cases revealed tachycardia, murmurs, gallop rhythm, muffled heart sounds and crackles in 68 dogs. Based on the severity of clinical signs, 80.77 % dogs were classified as “C2”, 16.67% as “C3”and 2.56% as “D” under the CHIEF system for prognostication and management of cases.

ECG abnormalities included arrhthymias and morphology changes of which sinus tachycardia and tall R waves were the most common respectively. Other abnormalities were by atrial fibrillation, ventricular premature complexes, atrial flutter, ventricular tachycardia, right bundle branch block, premature atrial contraction, tall R waves, tall T waves, short R waves, deep Q, deep S and ST segment sagging. Radiography was performed in 59 dogs and abnormalities included cardiomegaly, pulmonary edema, elevated trachea, decreased cardiophrenic angle, pleural effusion and ascites.

Hematology and routine biochemical results were within normal range except for a few outliers. Stress leukogram and mild anemia were observed along with azotemia in few cases. These tests were more useful in ruling out other diseases than diagnosing heart failure.

Biomarker evaluation indicated that human immunochromatography kit for cTnI is capable of detecting canine cTnI and was more sensitive than the canine specific sandwich ELISA kit of 0.156-10 ng/mL detection range. NT-proBNP sandwich ELISA kit was also

used and together with canine cTnI kit, 10 cases could be diagnosed as CHF out of 36 cases (samples).

Echocardiography was useful in diagnosis of CHF cases as either due to DCM or MVD by the measurements of E point to septal separation, fractional shortening, ejection fraction, mitral regurgitation, mitral valve degeneration and increased left atrium to aorta ratio.

Elevated levels of biomarkers were associated with increased severity of clinical signs, atrial fibrillation, DCM and azotemia and hence was of prognostic significance.

Therapeutic trials were undertaken by the addition of pimobendan and/ or nutraceutical to conventional therapy and the same were well tolerated and were found to be beneficial as evidenced by decreased mean scores for resolution of clinical signs. Even though the cost of these medications can be very high, especially for a large breed dog, the improvement in quality of life may justify it.

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VII. BIBLIOGRAPHY

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ABSTRACT

VIII. ABSTRACT

The present study was undertaken to study the occurrence of cardiac diseases in the dogs presented to Veterinary College, Bangalore and to evaluate the diagnostic/ prognostic potential of cardiac biomarkers like cardiac troponin I and amino terminal B-type natriuretic peptide. Diagnosis of congestive heart failure (CHF) due to mitral valve disease (MVD) or dilated cardiomyopathy (DCM) was done by history, physical examination, routine blood tests, electrocardiography, radiography and echocardiography. The occurrence of CHF was 0.45% with 76 % of dogs suffering from DCM and the rest 24% from MVD. Male dogs were more commonly affected than females. Dogs 5-10 years old were most commonly affected. The breeds most commonly affected were the Labrador Retriever (42.37 %), Non-descript dogs and Golden Retrievers with DCM and Pomeranian (42.12 %) and Non-descript dog with MVD. The Canine Heart failure International Expert Forum (CHIEF) system was followed for classification of the clinical cases. Common clinical signs were cough and ascites with tachycardia and murmurs often found on auscultation. Electrocardiogram commonly revealed sinus tachycardia, atrial fibrillation and tall R waves. Common radiographic findings were cardiomegaly and pulmonary edema. Hematology and biochemistry results were useful in ruling out the presence of other problems or diagnosing concurrent diseases. Cardiac troponin I and amino terminal pro-B-type natriuretic peptide canine specific ELISA tests were able to diagnose 10 out of 36 CHF cases and human immunochromatography kit was validated for canine cTnI and found to be sensitive. Echocardiography was useful in characterising cardiac diseases and quantifying the functional disturbances. Treatment trials with pimobendan and nutraceutical revealed that they were beneficial in the management of CHF cases, over the conventional protocol.

KEY WORDS: Dog, cardiac biomarker, echocardiography

APPENDICES

IX. APPENDICES

APPENDIX I

NT-proBNP analysis

i. Principle of the assay: The test employed was a sandwich ELISA technique.

ii. Reagents used:

- Assay plate: 12 x 8 antibody coated wells
- NT pro-BNP Standard
- Biotin antibody concentrate
- HRP avidin concentrate
- HRP avidin diluent
- Sample diluent
- Wash buffer
- TMB substrate
- Stop solution

iii. Equipments used:

- Microplate reader
- An incubator
- Squirt bottle
- Absorbent paper
- Micropipettes and pipette tips
- Test tubes for dilution

- Adhesive strips

iv. Reagent preparation

- All reagents were brought to room temperature for 30 minutes before use.
- Biotin antibody was diluted 100 fold by mixing 10 µL of the reagent with 990 µL of biotin antibody diluent.
- HRP- avidin reagent was diluted 100 fold by mixing 10 µL of the reagent with 990 µL of HRP- avidin diluent.
- Twenty mL of wash buffer (25x) was diluted with distilled water to form 500 mL of wash buffer (1x).
- The NT pro-BNP standard was reconstituted with 1 mL of sample diluent to obtain a stock solution of 20 ng/ mL. After 15 minutes of gentle agitation, two fold serial dilution of the standard was made to obtain 10, 5, 2.5, 1.25, 0.625 and 0.312 ng/ mL of standard solutions. The sample diluent was considered as blank/ zero standard.

v. Assay procedure

- The samples and standards were added to the wells of the assay plate at the rate of 100 µL per well and incubated for 2 hours at 37° C after covering the wells with adhesive strips to prevent evaporation.
- After 2 hours, the liquid from all the wells were removed.
- Hundred microliters of biotin antibody was added to all wells and again incubation was done at 37° C for 1 hour with the wells being covered with new adhesive strips.

- After 1 hour, the liquid in the wells was aspirated and the plate washed three times. After the last wash, the plate was inverted and blotted with clean paper towels.
- Hundred microliters of HRP-avidin was added to all wells and again incubation was done at 37° C for 1 hour with the wells being covered with new adhesive strips.
- The washing process was repeated as above for 5 times.
- TMB substrate was added to all the wells at the rate of 90 µL per well and incubated at 37° C for 15 minutes in a dark chamber.
- After 15 minutes, 50 µL of stop solution was added to all the wells and the optical density was read at 450 nm wavelength within 5 minutes.
- A graph was plotted using Curve Expert software and the values of NT-proBNP obtained.

Note: The test kits used in literature was first manufactured by the company Guildhay which then sold the patent on veterinary applications of NT-proBNP to IDEXX Laboratories in 2008. IDEXX Laboratories are now the sole worldwide manufacturer and supplier of NT-proBNP assays (Collins, 2013). Since this test kit is not available in India, a canine NT-proBNP kit from CUSABIO was procured. This could be one of the reasons why there is such disparity of results when values in literature were compared to those values in this study.

APPENDIX II

cTnI assay

i. Principle of the assay: The test employed was a sandwich ELISA immunoassay.

ii. Reagents used

- ELISA plate of anti cTnI-coated 96 wells
- cTnI Stock: Lyophilized dog cTnI (reconstitute with 0.40 ml H_2O)
- cTnI Diluent
- cTnI HRP Conjugate
- 20x Wash Solution
- TMB Reagent: HRP substrate solution
- Stop Solution: 1N HCl
- Distilled water

iii. Equipment used

- Micropipettes and pipette tips
- Micro-Plate incubator/shaker with mixing speed of ~150 rpm
- Microplate reader
- Vortex mixer
- Polypropylene microcentrifuge tubes (1.5 ml)
- Absorbent paper

iv. Preparation of reagents

- The wash solution (20x) stock was diluted prior to use by mixing the contents of the bottle (50 ml) with 950 ml of distilled water.
- Lyophilized cTnI stock was reconstituted by addition of 400 µL of distilled water and mixed gently several times over a period of 5 minutes. Serial dilution was made to obtain 10, 5, 2.5, 1.25, 0.625, 0.312 and 0.156 ng/ml of cTnI standard solutions.

v. Assay procedure

- cTnI HRP Conjugate (100 µL) was dispensed into each well.
- Standards and samples (100 µL) were also dispensed into appropriate wells.
- Incubatation was done on an orbital shaker (150 rpm) at room temperature (18-25°C) for 60 minutes.
- After incubation, the microtiter wells were emptied and washed 5 times with 1x wash solution.
- After washing, 100 µL of TMB reagent was added to each well and gently mixed for 5 seconds and incubated on an orbital shaker (150 rpm) at room temperature for 20 minutes.
- The reaction was stopped by adding 100 µL of stop solution to each well and gently mixed until all the blue color changed to yellow.
- Absorbance was read at 450 nm with a plate reader within 15 minutes.
- A graph was plotted using Curve Expert software and the values of cTnI obtained.

APPENDIX III

Normal value of canine cTnI: 0 to 0.08 ng/mL (Oyama and Solter, 2004)

Normal value of NT-proBNP: < 900 pmol/L (Collins, 2013)