STUDIES ON INFLUENCE OF RIGHT AND LEFT LATERAL RECUMBENCY ON VERTEBRAL HEART SCORE IN LABRADOR RETRIEVER DOGS

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CERTIFICATE

This is to certify that the thesis entitled ***"STUDIES ON INFLUENCE OF RIGHT AND LEFT LATERAL RECUMBENCY ON VERTEBRAL HEART SCORE IN LABRADOR RETRIEVER DOGS"*** submitted by **Mr. MAHADEV MULLATTI, ID. No. MVHK-1651** in partial fulfillment of the requirements for the award of degree of **MASTER OF VETERINARY SCIENCE** in **VETERINARY SURGERY AND RADIOLOGY** of the Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, is a record of bonafide research work carried out by him during the period of his study in this University under my guidance and supervision and the thesis has not previously formed the basis of the award of any degree, diploma, associate ship, fellowship or other similar titles.

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"Affectionately Dedicated To

All the Gurus of my life"

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Date : ***(Mahadev Mullatti)***

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LIST OF ABBREVATIONS/ SYMBOLS

%	Per cent
±	Plus or minus
≥	Greater than or equal to
≤	Lesser than or equal to
>	Greater than
<	Lesser than
viz.,	Namely
i.e.,	That is
ALT	Alanine aminotransferase
AST	Aspertate aminotransferase
BCS	Body condition score
BUN	Blood urea nitrogen
cm	Centimetre
cmm	Cubic millimeter
CDMD	Chronic degenerative mitral valve disease
CHF	Congestive Heart Failure
CK	Creatinine kinase
dL	Decilitre
DCM	Dilated cardiomyopathy
DLC	Differential Leukocyte count
DV	Dorso-ventral
ECG	Electrocardiogram
EDTA	Ethylene diamine tetra acetic acid
EDV	End systolic volume
EF	Ejection fraction
ESV	End diastolic volume
º F	Degrees Fahrenheit
Fig.	Figure
FS	Fractional shortening
g	gram
g/ dL	Gram per decilitre
Hb	Hemoglobin

IU/ L	International Units per Litre
IVSd	Interventricular septum during diastole
IVSs	Interventricular septum during systole
kg	Kilogram
KVp	Kilo voltage peak
L	Long axis
LDH	Lactate dehydrogenase
LL	Left lateral
L-VHS	Left lateral vertebral heart score
LVIDd	Left ventricular internal diameter during diastole
LVIDs	Left ventricular internal diameter during systole
LVPWd	Left ventricular posterior wall during diastole
LVPWs	Left ventricular posterior wall during systole
mg	Milligrams
mm	Millimetre
mmHg	Millimetres of mercury
min	Minute
MHz	Mega Hertz
MMVD	Myxomatous mitral valve disease/degeneration
MVD	Mitral valve disease/degeneration
mV	Millivolts
PCV	Packed cell volume
RL	Right lateral
R-VHS	Right lateral vertebral heart score
S	Short axis
SE	Standard error
sec	Seconds
T4	4^{th} thoracic vertebrae
TEC	Total erythrocyte count
TLC	Total leukocyte count
VHS	Vertebral Heart Score
v	thoracic vertebrae
VD	Ventro-dorsal

Introduction

I. INTRODUCTION

The modern lifestyle has made both human beings and dogs more susceptible to the cardiac disorders. Recent advances in Veterinary Cardiology, most notably in the areas of diagnostic imaging and cardiovascular therapeutics, have brought better understanding on cardiac disorders in dogs. Cardiac disorders during recent years were considered as an important health problems in dogs and were being diagnosed with an increased frequency (Devi *et al.*, 2009).

In clinical practice, dogs with cardiac diseases were generally diagnosed when they develop clinical signs or present with a clinical abnormality on examination. Diagnosis of cardiac diseases was made based on history, clinical findings, electrocardiographic, thoracic radiographic and echocardiographic examination.

Despite the availability of other diagnostic techniques like echocardiography (Root and Bahr, 2002), the thoracic radiography was one of the most commonly performed radiographic examination in small animal practice (Dark *et al.*,1996). Thoracic radiographs were important for assessing overall heart size, shape, pulmonary vessels, and lung parenchyma, as well as surrounding structures. It was the most commonly applied method for diagnosis of Congestive Heart Failure (CHF) and was considered the as "gold standard" diagnostic test (Balbarini *et al*., 1991). Earlier guideline of inter-costal spaces and cardio-thoracic ratio were used to evaluate the heart but due to some limitations such as thoracic confirmation, rib superimposition and imprecise measurement points, their usage was declined (Buchanan and Bucheler, 1995 and Gulanber *et al.*, 2005).

To overcome these limitations, a technique called Vertebral Heart Score (VHS) was evolved. The VHS is a method for objectively evaluating the dimensions of the cardiac silhouette in thoracic radiographs and was first described by Buchanan and Bucheler (1995). Cardiac silhouette refers to the outline of the heart as seen on lateral and ventrodorsal (VD) / dorsoventral (DV) thoracic radiographs and forms part of cardio-mediastinal contour. The size and shape of the cardiac silhouette provide useful clues for underlying disease. The measurement was based on cardiac height and width and was normalized to overall body size by comparison to vertebral body length. The mean VHS in different breeds of dogs reported by Buchanan and Bucheler (1995) was 9.7 ± 0.5 thoracic vertebrae (v) and 10.2 ± 0.83 thoracic vertebrae (v) vertebrae in lateral and VD thoracic radiographs respectively.

VHS is one of the easily available, applicable and interpretable cardiac diagnostic techniques as it does not require any sophisticated equipment. The VHS has proven to be reliable and repeatable method to calculate the cardiac silhouette with minimal inter-observer variability. However, the VHS measurements were affected by diverse cardiac confirmations between the canine breeds and the original reference range has been shown to be inaccurate for several breeds (Jaspen-Grant *et al.*, 2013). Therefore standardization of breed specific VHS will help in accurately diagnosing the anatomical cardiac disorders because there was comparable breed variation with respect to size and shape of normal heart among different breeds of dogs (Gugjoo *et al.*, 2013a).

Currently, various authors have different opinions on the effect of right lateral (RL) versus left lateral (LL) recumbency on VHS. Avner and Kirberger (2005) and

Mostafa and Berry (2017) affirm that right lateral recumbence provides the most accurate information on cardiac size, while Suter and Lord (1984) and Buchanan and Bucheler (1995) opined that, one lateral recumbency have no advantage over the other. Marin *et al.* (2007); Nabi *et al.* (2014) and Zahabpour *et al.* (2016) found no difference between views made in right versus left recumbency on the VHS assessment while Greco *et al.* (2008); Ghadiri *et al.* (2010) and Bodh *et al.* (2016) found significant difference between right lateral and left lateral recumbency on VHS.

Till now, various authors had given different VHS values for Labrador retrievers *i.e.*, Gugjoo *et al.* (2013a) reported 10.39 ± 0.05v and 10.29 ± 0.04v in right and left lateral recumbency respectively. Nabi *et al.* (2014) reported 10.21 ± 0.10v (RL), 9.93 ± 0.14v (LL), Neagu *et al.* (2015) reported 10.1v in right recumbency. Bodh *et al.* (2016) reported 10.39 ± 0.19v and 10.22 ± 0.22v in RL and LL radiographs respectively. Begum and Bhuvaneshwari (2018) reported 10.16 ± 0.36v in right recumbency.

The Labrador retriever is one of the most popular dog breeds in a number of countries in the world including India. These were frequently trained to aid those with blindness, act as therapy dog, or perform screening and detection work for law enforcement and other official agencies. This breed is best known for their obedience and loyalty. Labradors like to eat, therefore out of all the breeds these are most likely to become obese. Obesity predisposes them to various nutritional problems including the cardiac disorders (Raffan, 2013). Due to the over popularity and demand led to inbreeding of Labrador retriever dogs, they are becoming more vulnerable to genetic and developmental cardiac disorders. Development of breed specific VHS values particularly in breeds predisposed to cardiac disorders will improve accurate radiographic interpretation of cardiac silhouette (Birks *et al.*, 2017).

With this background the present study was conducted with the following objectives,

1. To record the occurrence of cardiac disorders among dogs.
2. Standardization of Vertebral Heart Score (VHS) values in normal Labrador retriever dogs in both right and left lateral recumbency by thoracic radiography.
3. To study the importance of VHS in diagnosing the cardiac disorders in dogs.
4. To correlate the VHS findings with electrocardiography and echocardiography.
5. To record clinical, physiological and haemato-biochemical parameters in dogs during period of study.
6. To record and compare the values of VHS in right and left lateral recumbency.

Review of Literature

II. REVIEW OF LITERATURE

2.1 Occurrence of cardiac disorders in dogs

Detweiler *et al.* (1968) reported that ten out of thousand dogs were commonly affected with different cardiovascular diseases.

Thrusfield *et al*. (1985) reported diagnosis of mitral insufficiency in 591 out of 16,357 dogs (3.6%) in their retrospective analysis of veterinary clinic records in Scotland.

Rush (2002) and MacPete (2018) estimated that approximately 10-15% of all dogs were affected with heart diseases.

Guglielmini (2003) opined that cardiac diseases are the second most prevalent cause of death in the dogs accounting for a percentage of 16.3.

Dukes-McEwan *et al.* (2003) reported that Dilated Cardiomyopathy (DCM) was the major cause of morbidity and mortality in various dog breeds.

Baumgartner and Glaus (2004) reported that prevalence rates of valvular disease in dogs as 49.40%, DCM (21.10%), pericardial effusiuon (12.4%) and hypertrophic cardiomyopathy (0.60%).

Deepti (2005) reported an overall prevalence of cardiac disease to an extent of 1.17% in a study on cardiac diseases and their management in dogs.

Atkins *et al.* (2009) reported that approximately 10% of dogs presented to primary care veterinary practices had heart disease, and chronic valvular heart disease was the most common heart disease of dogs in many parts of the world, accounting for approximately 75% of canine cases of heart disease.

Boghian and Mocanu (2012) reported the incidence of Congestive Heart Failure (CHF) in dogs as 7.03% (118 out of 1667). Among which 69.49% (82 cases) had left CHF, 8.47% (10 cases) had right CHF and 22.03% (26 cases) had global CHF.

Deepti (2015) reported that the occurrence of CHF in dogs was 0.45%, it was highest in Labrador Retriever dogs (25%) and more in 5-10 years age group.

Haritha *et al.* (2017) reported overall prevalence of cardiac disorders as 1.77% (113 dogs out of 6372) and it was 56.2% among (113 dogs out of 201) dogs exhibited clinical manifestation suggestive of heart disease.

Himalini *et al.* (2017) recorded the prevalence of cardiovascular disease in dogs as 1.61% (25 out of 1553) in their study on prevelance of cardiovascular diseases in canines of Jammu, and noticed left atrial enlargement to an extent of 40%, DCM (24%), CHF (16%), biatrial enlargement (12%) and secondary atrio-ventricular block (8%).

Badsar *et al.* (2018) reported the prevalence of cardiac abnormalities to an extent of 2.41% (40 out of 1655) and amongst the suspected cases for cardiac disease, it was 40.81% (40 out of 98).

Favril *et al.* (2018) reported from a review on Tricuspid valve dysplasia in dogs that it represents approximately 2 to 7 % of all congenital malformations.

Mounika *et al.* (2020) reported obesity in 732 (31.39%) dogs out of 2,332 dogs that were screened during the study period. Out of 732 obese dogs, 171 (23.36%) showed the cardiovascular problems.

2.1.1 Age-wise occurrence of cardiac disorders in dogs

Calvert *et al.* (1997) reported from several retrospective studies on Doberman pinscher breeds and their relation with DCM that median age of dogs with DCM was ranged between four to eight years.

Sisson *et al.* (2000) reported that the prevalence of DCM was 0.16% in mixed breeds where as 0.65% in purebred dogs. The median age of dogs with DCM was between 4 to 8 years.

Guglielmini (2003) reported higher prevalence of DCM in middle and old aged dogs.

Deepti (2005) reported the highest occurrence (54.90%) of cardiac diseases in 8 to 12 years age group dogs irrespective of breeds.

Detweiler and Patterson (2006) reported that the prevalence of murmur was 11%, 24% and 37% respectively in dogs aged 5 to 8 years, 9 to 12 years and 13 years or over.

Yathiraj (2007) reported that the heart worm infection was common in dogs of three to eight years of age.

Martin *et al.* (2009) reported from study on 369 cases of DCM that majority of dogs were presented at six to eight years of age.

Wess *et al.* (2010) reported the occurrence of cardiovascular diseases in Doberman Pinschers as the highest occurrence was in dogs aged more than 6 years (88.7%), followed by dogs aged between 2 and 6 years (22.4%) and the least common in young dogs up to 2 years (3.3%).

Chetboul and Tissier (2012) reported in their study on echocardiographic assessment of canine degenerative mitral valve disease that occurrence of Mitral Valve Disease (MVD) was 14-40% in small sized dogs and which was higher in geriatric dogs.

Mourya *et al.* (2018) reported cardiac diseases were often a diagnostic problem and determination of the heart size was important for evaluation the patient with cardiac disease. Cardiomegaly was one of them and often seen in older dogs as compared to younger ones.

2.1.2 Gender-wise occurrence of cardiac disorders in dogs

Lombard (1984) reported that idiopathic dilated cardiomyopathy occurs commonly in large dog breeds and males of the breeds.

Hesslink (1988) reported that the prevalence of heart worm infestation in male dogs was significantly higher (12.8%) than in females (7.2%).

Swenson *et al*. (1996) reported the prevalence and intensity of heart murmurs was higher among males than among females of 5 year old Cavalier king charles spaniels.

Calvert *et al.* (1997) reported that males were affected nearly as often as females in their retrospective study on Doberman pinscher dogs with heart failure which was attributed to DCM.

Myxomatous Mitral Valve Disease (MMVD) causes mitral valve insufficiency which was manifested clinically by a left apical holosystolic murmur. The prevalence of such murmurs increases with age occurs more commonly in certain breeds and more frequently in males (Haggstrom *et al.*, 1995; Haggstrom, 1996 and Pedersen *et al.*, 1999).

Vollmar (2000) reported that the prevalence of cardiomyopathy in the Irish wolfhound breed of dogs as 59.8% (299/500) in females and 40.2% (201/500) in males.

Dukes-McEwan *et al.* (2003) reported that the overall prevalence of canine idiopathic DCM was 0.5% according to Purdue University records. Breed prevalence was higher in pedigree dogs with male dogs showing an early onset.

Detweiler and Patterson (2006) reported that a gender difference, appeared to become more and more obvious with increasing age. Gender differences also have been reported in the occurrence of congestive heart failure due to mitral regurgitation, and its prevalence was 4.5 times higher in male than in female dogs.

Martin *et al.* (2009) reported that DCM occurred primarily in medium to large breed pure-bred dogs, and males were more frequently affected than females.

Stephenson *et al.* (2012) reported that prevalence of DCM in Great Dane breed of dogs was highest in females (61.16%) than males (38.84%) in their study on 103 dogs.

Rajkumar (2013) reported in a retrospective study on cardiac diseases in 184 dogs that the occurrence was more in male dogs *i.e.*, 126 (68.5%) as compared to female ones *i.e.*, 58 (31.5%).

Haritha *et al.* (2017) reported the highest prevalence of cardiac disorders in male Labrador retriever breed of dogs aged between five to ten years and the lowest in female Dachshund breed of dogs aged less than five years in their work on prevalence of cardiac disorders in canines.

2.1.3 Breed-wise occurrence of cardiac disorders in dogs

Soares *et al.* (2010) reported that DCM was the second most common cause of cardiac morbidity and mortality in dogs, mainly among large and giant breeds in their study on survival and echocardiographic evaluation of dogs with idiopathic dilated cardiomyopathy treated with carvedilol.

Braslasu *et al.* (2013) reported that tricuspid degenerative valvulopathy was commonly diagnosed in small breed dogs as compared to large and giant breed dogs.

Himalini *et al.* (2017) reported highest prevalence of cardiac diseases in Labrador retriever (44%) and lowest in Cocker spaniel, Saint bernese, Pug, Bullmastiff, Lhasa apso, Doberman and Bakerwali *i.e.*, 4% each in their study on prevalence of cardiovascular diseases in canines of Jammu.

Reetu *et al.* (2017) and Hoque *et al.* (2019) reported in their retrospective study that the highest incidence of cardiac disease in dogs was of systolic dysfunction followed by DCM and congestive heart failure. Maximum incidences were seen in Labrador retriever and in male dogs and geriatric dogs were found to be mostly affected.

Mourya *et al.* (2018) reported that giant breed dogs often develop DCM. Pericardial effusion, aortic effusion and endocarditis seem to be more frequently developed in large breed dogs.

Jeyaraja *et al.* (2019) reported that the incidence of DCM in Labrador retriever dogs was 7.49% in his study on retrospective analysis of DCM in Labrador retrievers for a period of five years from 2013 to 2018.

2.2 Diagnosis

Detweiler *et al.* **(**1961) reported that auscultation, electrocardiography and roentgenography were the three most useful diagnostic procedures employed in recognizing the heart diseases in dogs.

Taylor and Sittnikow (1968) used auscultation, roentgenograms and electrocardiograms in diagnosing cardiac diseases in dogs, and reported that careful auscultation was the most important method of diagnosing cardiac disease but confirmation of cardiac disease and evaluation of its severity can be best obtained through the use of the roentgenogram and electrocardiogram.

Nakayama *et al.* (2001) reported that various diagnostic tools for the diagnosis of heart disease were available that include: clinical examination and auscultation, thoracic radiography, electrocardiography, echocardiography and cardiac biomarkers.

2.2.1 Clinical signs and physical examination

Atkins (1994) reported that in the presence of severe left atrial enlargement due to mitral valve insufficiency without heart failure, cough commonly has been anecdotally attributed to compression of the left main stem bronchus by a large left atrium.

Although the presence of a holosystolic murmur was a good indicator of the presence of the disease, it should be noted that dogs with mild disease may not have a murmur, even though they had echocardiographic evidence of MVD and mitral regurgitation (Haggstrom, 1996 and Pedersen *et al*., 1999).

Dukes-McEwan *et al.* (2003) reported that the clinical signs and physical examination findings in cardiac diseases included exercise intolerance, weakness, cyanosis, syncope, pale mucous membrane, weak femoral pulse, pulse deficit, cough, dyspnoea, tachypnoea, ascites, tachycardia, arrhythmia, polydipsia, inappetance, weight loss, muscle wasting and elevated body temperature in their study on diagnosis of canine idiopathic dilated cardiomyopathy.

Ristic (2004) reported that cough was the most common clinical sign reported by the owner and tends to be soft with cardiac disease, honking with tracheal disease and harsh and sometimes productive with bronchial disease.

Martin *et al*. (2009) reported in a retrospective study that most common clinical findings in canine dilated cardiomyopathy were weak pulse (39 per cent), murmur (33 per cent), mucosal pallor (16 per cent), ascites (15 per cent) and a gallop sound (10 per cent).

Devi *et al.* (2009) reported in a study that most of the clinical cases of cardiac diseases were presented with a history of nocturnal coughing (2.55%), exercise intolerance (1.82%), partial or complete anorexia (1.82%), swelling in abdominal area (1.45%), dullness and depression (0.72%), cachexia and jugular pulsation (0.36% each) at times.

Singh *et al.* (2012) studied bronchomalacia in dogs with myxomatous mitral valve degeneration. Their results failed to identify an association between left atrial enlargement and airway collapse in dogs with MVD, but did suggested that airway inflammation was common in dogs with airway collapse.

Mourya *et al.* (2018) reported dry coughing, increased on exercise and were not responding to therapy as clinical signs in dogs with cardiomegaly.

Rajamohan *et al.* (2018) reported fluid thrill on percussion of distended abdomen, tachycardia, weak femoral pulse and dyspnoea as clinical findings in Labrador retriever dog with DCM.

Mounika *et al.* (2020) reported exercise intolerance and cyanosis on exertion in obese dogs affected with cardiovascular disorders.

2.2.2 Clinical parameters

2.2.2.1 Body condition score (BCS) and Body weight

Laflamme (1997) stated that the BCS included a number of categories, ranging from emaciated to severely obese, based on subjective assessment of specific features, which included the shape of the animal when viewed from the above and how easily ribs were palpable. Similar opinions were also reported by Toll *et al.* (2010) and Sandoe *et al.* (2014).

Kuruvilla and Frankel (2003) reported that obesity increases risk of death from all causes, was linked to increased risk of development of hypertension and negatively impacts cardiovascular and pulmonary functions.

Devi *et al.* (2009) reported that obesity and heart diseases in dogs were encountered frequently in clinical practice. Improper feeding, inadequate exercise and managemental negligence favour obesity that may leads to heart diseases. A variety of nutritional deficiencies- dietary protein, fat, vitamins, minerals and trace elements were known to cause cardiac disease in various species.

Goutal *et al.* (2010) found no association between cardiac disease occurrence and animal survival chances with different BCS in a study on evaluation of acute CHF in dogs and cats.

Castro *et al.* (2011) reported a significant correlation between the VHS and body weight on lateral and ventro-dorsal radiographs in normal Yorkshire Terrier dogs having a homogeneous sample weight and age.

Gugjoo *et al.* (2013a) reported that the vertebrae and internal organ size show a comparable development and can be attributed to non-significant effect of body weight on vertebral heart score.

Jaspen-Grant *et al.* (2013) studied vertebral heart score in eight dog breeds (Pug, Pomeranian, Yorkshire terrier, Dachshund, Bulldog, Shih Tzu, Lhasa Apso, and Boston terrier dog breeds) and reported that VHS of Lhasa Apso was significantly affected by BCS and this could be because of possible variations in the amount of pericardial fat in different breeds.

Manens *et al.* (2014) reported that the weight loss was associated with improvement in cardiovascular and respiratory function in obese dogs.

Thengchaisri *et al.* (2014) reported that the average BCS was comparable between healthy dogs and dogs with heart disease, but the difference was not significant. Furthermore, the abdominal obesity was associated with heart diseases in dogs rather than overall obesity.

Bodh *et al.* (2016) reported that the body weight did not correlated significantly with VHS in Spitz, Labrador retriever and Mongrel dogs.

Mounika *et al.* (2020) reported that weight reduction alleviate the signs of cardiovascular diseases and would improve the quality of life, provides evidence that weight reduction approaches in canine practice should be given the utmost importance and there is outright need for maintaining canines in ideal body weight.

Puccinelli *et al.* (2020) reported that there were non-significant differences in VHS, body condition score and body weight in his study on Chihuahua breed of dog.

Taylor *et al.* (2020) reported that the Norwich terriers with BCS > 6 had significantly higher VHS values than those with BCS < 5.

2.2.3 Physiological parameters

Dillon and Brawner (1995) noticed a rise in temperature (mild to moderate) in dogs affected with venacaval syndrome caused by Dirofilariasis.

Tidholm and Jonsson (1997) reported tachycardia on cardiac auscultation in dogs with DCM.

Montoya *et al.* (2006) and German *et al*. (2010) reported that obesity in dogs was associated with an increased risk of high blood pressure and cardiac diseases.

Bomassi (2007) reported an increased respiratory rate (35 breaths/minute), high heart rate (180-190 beats/ minute) in Saluki breed of dogs with advanced mitral valve disease.

Devi *et al.* (2009) reported that high fat diet induces abdominal obesity in dogs and resulted in significantly high blood pressure and heart rate, thus may lead to heart disease due to atrial hypertension and left ventricular hypertrophy.

Schneider *et al.* (2011) reported tachycardia in juvenile DCM affected Labrador retriever.

Bodh *et al.* (2014) reported normal rectal temperature, heart rate, respiratory rate and blood pressure in Labrador retriever dogs with occult DCM. They also reported elevated lactate dehydrogenase (LDH), elevated creatinine kinase-MB (CK-MB) and aspartate aminotransferase (AST) in the same dogs.

Sanchez *et al.* (2015) reported that obesity was not a risk factor to develop hypertension and the hypertension present in dogs was related to co-morbidities such as chronic kidney disease, cardiomyopathies and endocrinopathies.

Kanno *et al.* (2016) noticed a significant increase in heart and respiration rates in right sided CHF dogs when compared to healthy control dogs and non CHF dogs

Davis *et al.* (2017) reported that brachycephalic dogs had greater increase in respiratory rate in response to heat stress than that of non-brachycephalic dogs.

2.2.4 Haemato-biochemical parameters

Tidholm and Jonsson (1997) reported in a retrospective study on canine DCM in 189 dogs that no abnormalities in the majority of dog's routine biochemical analysis and haematology except mild hyperglycemia and mild to moderate hypercholesterolemia in 38% and 33% of cases, respectively.

Dunn *et al.* (1999) reported that serum electrolyte concentrations were often in the normal range, with modestly elevated serum liver enzymes (AST and ALT), serum urea nitrogen, creatinine and creatinine kinase in dogs with DCM.

Kaneko *et al.* (1999) reported that in severe cardiac failure there was evidence of azotaemia with low total protein and albumin. Elevated ALT and serum alkaline phosphatase were noticed in animals with right sided congestive cardiac failure.

Hypokalemia, hypochloremia and hyponatremia were noticed in dogs with cardiac failure.

Sisson and Kittleson (1999) reported that the complete blood count of dogs with DCM was same as the normal dogs except for a moderate lymphopenia and occasionally to modest neutrophilia.

Sisson *et al.* (2000) reported that complete blood count was useful to detect other concurrent diseases and to guide the therapy and observed modestly elevated serum urea nitrogen and creatinine in dogs with DCM.

DeMorais (2000) reported that haematology and biochemistry were not useful for the diagnosis of heart diseases; however, they could be helpful to investigate potential concurrent disease.

Stafford *et al.,* (2004) reported haematological and biochemical findings obtained in 85 cases with pericardial effusion. Anaemia with haematocrit below 37 per cent was found in 34 cases (40 per cent). There was no difference in the prevalence of anaemia between echo-positive and echo-negative cases. Total protein concentrations below 53 g/litre were found in 19 cases (22 per cent). Albumin concentrations below 24 g/litre were found in 14 cases (16 per cent) and globulins below 26 g/litre were found in 38 cases (45 per cent). Again, no difference in these parameters was apparent between echo-positive and echo-negative dogs.

Boswood and Murphy (2006) recorded that there was non-significant alterations in red blood cell count or haematocrit in advanced stages of heart failure in 92 dogs. They also reported that decreased serum sodium concentration indicated in more advanced stages of heart failure. They further added that creatinine and blood urea concentrations changed significantly according the heart failure category and changes reflected the transition from heart disease to heart failure.

Glinska *et al.* (2006) noticed the leucocytosis in ten severe right sided heart failure dogs in their study on histopathological changes in right heart failure in dogs. They noticed increased urea levels in ten severe heart failure dogs.

Sesh *et al*. (2013) reported the occurrence of decrease in haematocrit values with overall blood picture indicative of ischemia and acute infection in dogs with DCM.

Rajamohan (2018) reported haematological parameters were within normal range and biochemical parameters like total proteins and albumin were mildly below the normal range in Labrador retriever dog with DCM.

2.2.5 Diagnostic parameters

2.2.5.1 Thoracic radiography

Toombs and Ogburn (1985) reported that radiographic diagnosis of canine cardiac disease was based on recognition of signs, including abnormal size and shape of the cardiac silhouette, abnormal size or shape of the pulmonary vessels, presence of pulmonary oedema and ascites. In dogs that were not in congestive cardiac failure, the radiographic examination of cardiac disease was focused on the evaluation of the size and shape of the cardiac silhouette; increased size was an expected result of cardiac diseases that lead to dilatation or eccentric hypertrophy, while abnormal shape may occur as a result of enlargement of one or more cardiac chambers therefore, its recognition may aid in diagnosis of certain specific cardiac conditions.

Balbarini *et al.* (1991) reported that thoracic radiographs were important for assessing overall heart size and shape, pulmonary vessels, and lung parenchyma, as well as surrounding structures. It was the most commonly applied method for diagnosis of CHF and was considered as the gold standard diagnostic test.

Dunn *et al.* (1999) reported that thoracic radiographs of some breeds like Doberman pinscher and Boxer suffering from DCM appeared remarkably normal cardiac silhouette with mild left atrial and left ventricular enlargement. The increased convexity and increased sternal contact on lateral radiographic projections were signs of pure right sided cardiomegaly caused by pulmonic stenosis, tricuspid regurgitation, pulmonary hypertension or heart worm disease.

Sisson *et al*. (2000) reported that in cardiomegaly, cardiac silhouette appears rounded with clear demarcation of contour of heart chambers, while in pericardial effusion cardiac contours get obliterated due to accumulation of fluid within the pericardial sac.

Kirsch *et al.* (2000) and Johnson *et al.* (2004) reported that thoracic radiography was an insensitive means in diagnosing pericardial effusion because pleural effusion would mask the cardiac silhouette which made the presumptive radiographic interpretation difficult.

Lamb *et al.* (2001) concluded that survey radiography was an inaccurate method for diagnosis of canine congenital cardiac anomalies because of the difficulty of recognising radiographic signs, which were not present in many cases.

Root and Bahr (2002) reported that alteration in the shape and size of cardiac silhouette, abnormal size, shape of pulmonary vessels and the presence of pulmonary edema or ascites on thoracic radiographs were often the hallmarks of radiographic diagnosis of cardiac diseases in dogs. They recorded left atrial enlargement, left ventricle enlargement, pulmonary edema in left sided heart failure; pulmonary venous congestion and pleural effusion in right sided heart failure in canine thoracic radiographs with generalized cardiomegaly.

Radiographic diagnosis of cardiac disease included an assessment of the size and shape of the cardiac silhouette (Lamb and Boswood, 2002).

Stafford *et al.* (2004) carried out retrospective study in 143 dogs with pericardial effusion. A total of 90 dogs underwent thoracic radiography. Cardiomegaly with a globular cardiac silhouette evident in 78 cases (87 per cent) and pleural effusion was present in 31 cases (34 per cent).

Hansson (2004) studied the imaging of the left atrium and described the enlarged left atrium as a bulge in the caudo-dorsal cardiac contour and an elevation of the distal part of trachea towards the thoracic spine.

Gidlewski and Petrie (2005) stated that the margins of the cardiac silhouette depends on the chronicity of the disease in their study on pericardiocentesis in dogs and cats.

Bomassi (2007) reported the radiographic observations like bilateral cardiomegaly with considerable increase in size of left atrium, decreased trachea vertebral angle, pulmonary venous densities and alveolar pulmonary oedema, consistent with congestive disease in a dog with severe MVD.

Martin *et al.* (2009) studied thoracic radiograph in canine cardiomegaly and reported that cardiomegaly was evident in 80% and congestive signs (pulmonary oedema or pleural effusion) in 74%.

Hansson *et al.* (2009) reported that radiography was of unspecified sensitivity and specificity, especially in the setting of combined heart and lung disease, and can suffered from considerable observer variation.

Thoracic radiography had an important role in the evaluation of the cardiovascular system (Lord *et al.*, 2011 and Bahr, 2018)

Guglielmini *et al.* (2012) reported that the characteristic radiographic sign of pericardial effusion in dogs was an enlarged cardiac silhouette with globoid shape, due to stretching of pericardium via accumulation of pericardial fluid.

Buchanan (2013) opined that radiography was useful tool for diagnosis of cardio-pulmonary disease in small animals by providing reliable evidence of heart size and altered contours and pulmonary changes.

Gugjoo *et al.* (2013a) reported that the radiographic interpretation of heart can be done by number of ways *viz.*, gross examination or by using different measurements like cardiothoracic ratio, relationship with intercostal spaces or Vertebral Heart Score (VHS).

Baisan *et al.* (2017) opined that the heart should be considered radiographically normal unless there was an obvious change in size or shape. However, a radiographically normal heart does not exclude a cardiac disease. It was important to consider the breed and size when performing measurements of thoracic radiographs to avoid any error of the interpretation for cardiac enlargement. Cardiac measurement was useful for general overview after cardiac silhouette and for the dynamic progression of the disease. Thoracic radiography was a complementary exam and it must be used along with echocardiographic exam for a certain diseases of valves.

Mourya *et al.* (2018) reported that thoracic radiograph was very helpful in the diagnosis of heart disease and it provides immediate information about the size, shape and position of the heart and its relation to adjacent structures and was therefore of paramount importance for understanding of the heart diseases.

2.2.5.1.1 Vertebral Heart Score (VHS)

2.2.5.1.1.1 History and development

Buchanan and Bucheler (1995) first described the Vertebral Heart Score (VHS) as method of objective evaluation of cardiac silhouette on thoracic radiograph and reported the mean VHS values of different breeds of dogs to be between 9.7 ± 0.5 and 10.2 ± 0.83 thoracic vertebrae in lateral and ventro-dorsal radiographs respectively and these values were unaffected by depth or broadness of the chest of dogs. They developed this technique to overcome the limitations in terms of, variations in the axis of the heart and its silhouette, thoracic conformation, respiratory phase, rib superimposition and imprecise measurement points present in earlier techniques such as a guideline of 2.5 to 3.5 5 intercostal spaces for dogs with a deep and wide thorax respectively (Owens, 1985 and Kealy, 1987) and cardio-thoracic ratios (Toombs and Ogburn, 1985). This technique involved the measurement of heart:skeletal ratios and was anatomically justifiable, reasonably precise and simple to use and explain. This technique was developed on the basis of comparison between the heart size and vertebral length, because both were measurable in thoracic radiographs and good correlation known to exist between heart weight and body length.

2.2.5.1.1.2 Technique

Buchanan and Bucheler (1995) employed measuring the cardiac silhouette by involving its long axis (taken from left main stem bronchus ventral border to the cardiac apex with a measuring scale) and short axis (taken from central third region of heart perpendicular to the longitudinal axis with a measuring scale) on a lateral thoracic radiograph. The sums of these measurements were then compared to the mid-thoracic vertebral bodies starting from the anterior edge of the fourth thoracic vertebra (T4). Summation of number of vertebrae in relation to long and short axis of heart indicates Vertebral Heart Score (VHS). They reported that precise measurements for statistical analysis were taken to the margin of 0.1 vertebrae and a l0-vertebrae-long index of body length was estimated by doubling the length of 5 vertebrae from the cranial edge of T4 to the caudal edge of T8. In VD and DV radiographic views, the maximal long and short axes of the heart were determined with callipers in similar fashion and measured against vertebrae in the lateral radiographic view beginning with cranial edge of T4. They also reported that long axis of heart includes left atrium and left ventricle in lateral radiograph and right atrium and left ventricle in dorso-ventral radiograph. The short axis of the heart includes right atrium and left heart chambers in lateral radiograph and left and right heart structures in dorso-ventral radiograph.

2.2.5.1.1.3 Breed

Toombs and Ogburn (1985) and Root and Bahr (2002) reported that there was considerable breed variation with regards to normal heart size and shape. So, it was desirable to consider the breed specific value whenever the heart was evaluated.

Buchanan and Bucheler (1995) reported that the breeds with long thorax had lower VHS value (Dachshund, 9.5v) and those with short thorax had higher VHS value (Miniature Schnauzer, 11v).

Lamb *et al.* (2001) reported that Boxer dog breed had significantly higher mean VHS measurements as compared to the dogs of other breed including Yorkshire terrier and German shepherd while Labrador retrievers had significantly higher mean VHS measurements than other breeds except the Boxer and the Cavalier King Charles spaniels and reported VHS value of Labrador retrievers to be 10.8 ± 0.6v in right lateral recumbency. He also reported that higher VHS in Labrador retriever, Boxer and Cocker spaniels as compared to other breeds might be due to slightly shorter vertebrae in such breeds.

Fonsecapinto and Iwasaki (2004) reported the Vertebral Heart Size (VHS) value in normal Poodle breed of dogs to be smaller or equal to 10.5 in 80% of dogs.

Marin *et al.* (2007) reported the mean VHS established on lateral radiographs was 10.5 ± 0.1 for Greyhounds, 9.8 ± 0.1 for Rottweiler and 10.1 ± 0.2 for mixed breed dogs.

Kraetschmer *et al.* (2008) reported VHS in the Beagle dogs *i.e.,* 10.3v which was significantly different from the mean value of 9.7v shown in Buchanan and Bucheler (1995) study.

Ghadiri *et al.* (2010) reported the VHS in Doberman, German shepherd and other native breed of dogs in Iran on both LL and RL views as 9.9v and 10.1v, 9.6v and 9.8v, and 9.6v and 9.7v respectively. They reported that the mean VHS values of Doberman and German shepherd in LL views were significantly lower than RL views and non-significant difference was observed in native breed of dogs.

Lahm *et al.* (2011) reported the mean value of VHS in American pit bull Terrier *i.e.,* 10.9 ± 0.4v

Gugjoo *et al.* (2013a) reported VHS values in Labrador retriever breed of dog *i.e.*, 10.39 ± 0.05v and 10.29 ± 0.04v in RL and LL respectively. They recorded the mean ± SE values for short axis as 4.66 ± 0.02v and 4.67 ± 0.04v and long axis as 5.65 ± 0.03v and 5.75 ± 0.03v on both left and right lateral recumbency respectively.

Jaspen-Grant *et al.* (2013) reported VHS in eight dog breeds (Pug, Pomeranian, Yorkshire terrier, Dachshund, Bulldog, Shih Tzu, Lhasa apso, and Boston terrier dog breeds) and they opined that the VHS in Pug, Pomeranian, Bulldog, and Boston terrier was significantly greater than 9.7 ± 0.5v.

Nabi *et al.* (2014) reported the VHS values for some breeds of India *viz.*, Labrador retriever as 10.21 ± 0.10v (RL), 9.93 ± 0.14v (LL) and 10.09 ± 0.13 (DV), German shepherd as 10.24 ± 0.35v (RL), 10.03 ± 0.27v (LL) and 10.30 ± 0.42v (DV), Indian spitz as 9.63 ± 0.23v (RL), 9.46 ± 0.39v (LL) and 9.79 ± 0.35 (DV), Pug as 10.09 ± 0.22v (RL), 9.53 ± 0.33v (LL) and 10.03 ± 0.34 (DV) and for non-descript dogs as 9.59 ± 0.31v (RL), 9.39 ± 0.45v (LL) and 9.42 ± 0.45 (DV) in their study on radiographic measurements (vertebral heart scale) of popular breeds of dogs in India.

Srivastava *et al.* (2014) reported the VHS in healthy Mongrel dogs as 10.13 ± 0.18v with range of 8.9 to 11.5v. They reported the mean ± SE values for short axis and long axis as 4.57 ± 0.10v and 5.55 ± 0.09v respectively.

Neagu *et al.* (2015) recorded the VHS in different breeds of dogs *viz.*, Pekingese as 9.2 (8.7 to 10.4v), Bichon frise as 9.2 (8.7 to 10.7v), German shepherd as 9.8 (9.1 to 10.5v), Labrador retrievers as 10.1 (9.6 to 11v), German brack as 10.9 (10.5 to 11.7v) and

Mongrel as 9.5 (8.8 to 10.5v) in right lateral recumbency from study on 90 dogs which had 15 animals in each breed. They concluded that VHS was influenced by conformation of thorax and it was recommended to consider the status of the individuals, age, gender and breed for VHS measurements.

Bodh *et al.* (2016) reported that the Labrador retrievers had the highest VHS in LL and RL radiographs (10.22 ± 0.20v and 10.39 ± 0.19v, respectively) followed by Spitz (10.03 ± 0.11v and 10.21 ± 0.13v, respectively) and Mongrel dogs (9.62 ± 0.25v and 9.82 ± 0.21v, respectively). He added that LL and RL VHS in Spitz and Labrador retriever was significantly greater than reference VHS of 9.7 ± 0.5v.

Begum and Bhuvaneshwari (2018) reported that the VHS values in Dachshund, Doberman pinscher, German shepherd, Golden retriever, Labrador retriever, Mongrel, Pug, Rottweiler, Shih Tzu and Spitz breed of dogs as follows 9.45 ± 0.52, 10.06 ± 0.76, 10.21 ± 0.55, 9.52 ± 0.62, 10.16 ± 0.36, 9.86 ± 0.71, 10.57 ± 0.55, 9.83 ± 0.37, 9.56 ± 0.70 and 9.22 ± 0.56 respectively. They reported that the result showed significant difference between breeds.

Mourya *et al.* (2018) considered those dogs having VHS of > 9.7 ± 0.5v were considered as cardiomegaly. The long (L) and short (S) axis for Labrador L = 7 and S = 6 and for Mongrel L = 7 and S = 5 and sum of the long and short axis of the heart was 13v and 12v respectively as compared to normal VHS of 9.7v (8.5 – 10.5v).

Puccinelli *et al.* (2020) stated that the Chihuahua breed had a greater VHS (10.0 ± 0.6v) on lateral thoracic radiographs than the canine reference value of 9.7 ± 0.5v established by Buchanan and Bucheler. Conversely, the mean VHS on VD thoracic radiographs in their study (10.4 ± 0.5) did not differ from the value of 10.2 ± 0.8v as proposed by Buchanan and Bucheler.

Taylor *et al.* (2020) reported that the VHS value of Norwich terriers with no clinical signs of cardiovascular disease of 10.6v was found to be significantly greater than the canine reference value of 9.7 ± 0.5v initially established by Buchanan and Bucheler.

2.2.5.1.1.4 Age

Sleeper and Buchanan (2001) reported that VHS of puppies does not change significantly with the growth to three years of age and lies within adult dog's reference range (9.7 ± 0.5v). Thus, similar standards existed to determine cardiac enlargement in puppies and adult dogs.

Gulanber *et al.* (2005) reported that age did not significantly influence VHS values in Turkish shepherd (Kangal) dogs.

Zahabpour *et al.* (2016) reported that age, weight and respiratory phase showed no significant relation with VHS in native dog in Khorasan province, Iran.

Birks *et al.* (2017) studied the influence of age on VHS in 51 normal Dachshund breed of dogs and reported the probable cause for increase in VHS values with increasing age in their study could be due to increased epicardial fat deposition with advancement of age.

Begum and Bhuvaneshwari (2018) reported that there was non-significant difference of VHS among age groups of the same breed of dogs.

Taylor *et al.* (2020) reported that there was no significant effect of age on VHS values in Norwich terriers.

2.2.5.1.1.5 Gender

Buchanan and Bucheler (1995) reported that gender did not significantly influence VHS values in dogs.

The method of quantifying cardiomegaly by VHS has been used frequently. This method has been shown to be relatively easy to perform and produces results independent of thoracic dimensions and gender (Sammarco *et al.*, 1995 and Kirberger and Lobetti, 1998).

Bavegems *et al.* (2005) reported that gender did not significantly influence VHS values in Whippets.

Gulanber *et al.* (2005) reported that gender did not significantly influence VHS values in Turkish Shepherd (Kangal) dogs.

Ghadiri *et al.* (2010) reported that gender did not significantly influence VHS values in Doberman, German shepherd and other native breed of dogs in Iran.

Gugjoo *et al.* (2013a) reported that gender did not significantly influence VHS values in Labrador retriever dogs.

Srivastava *et al.* (2014) reported the non-significant variation between the male and female dogs in relation to VHS, long axis and short axis in mongrel dogs.

Bodh *et al.* (2016) reported that there was non-significant differences in the VHS between male and female dogs within Labrador retriever, Indian spitz and Mongrel breeds.

Birks *et al.* (2017) reported that the VHS for females was significantly larger than males (left: 10.56 versus 9.74 and right: 10.8 versus 9.99) in Dachshunds.

Begum and Bhuvaneshwari (2018) reported that there was no significant difference of VHS among gender of the same breed of dogs.

Taylor *et al.* (2020) reported that there was no significant effect of sex on VHS values in Norwich terriers.

Puccinelli *et al.* (2020) stated that the gender did not significantly influence the VHS values in Chihuahuas.

2.2.5.1.1.6 Positioning

Buchanan and Bucheler (1995) reported on comparison between DV and VD thoracic radiographic views and revealed that VD heart sizes were wider (7%) and longer (5%) than DV heart sizes. DV radiographic views were preferred over VD views for evaluation of heart size because cardiac contours were more consistent in DV view and there was magnification in VD views caused by increased distance between the heart and the cassette.

Fox (2003) first studied the effect of radiographic positioning on VHS in dogs and reported that lateral radiographic positioning in clinical practice may be preferred over VD as it is less stressful for cardiac patients.

Gulanber *et al.* (2005) reported that image magnification occurs in VD views as the distance between heart and the X-ray cassette increases. In addition to magnification, higher VHS may occur as the VD/DV long axis includes the right atrium and left ventricle, whereas in lateral projections only the left atrium and left ventricle were included.

Bavegems *et al.* (2005) reported higher VHS in RL recumbency than in LL recumbency in Whippets.

Marin *et al.* (2007) reported non-significant effect of radiographic positioning (right or left recumbency) on VHS in Greyhounds, Rottweiler and mixed breed dogs.

Kraetschmer *et al.* (2008) reported that VHS values of Beagle dogs were higher on right lateral recumbency as compared to left lateral recumbency and explained that the position of heart within the thorax changes slightly as a result of gravity when the animal was restrained in different recumbency leading to change in the VHS

Greco *et al.* (2008) compared VHS calculated in right and left lateral recumbency and reported significantly higher VHS value in right (9.8 ± 0.6v) as compared to left lateral recumbency (9.5 ± 0.8v) in normal dogs.

Ghadiri *et al.* (2010) reported the findings in common large breed dogs of Iran showed non-significant difference in VHS when taken in right or left lateral recumbency.

Gugjoo *et al.* (2013a) reported that determination of VHS in DV or VD projection in deep chested dogs appeared to be of little value as there was relatively vertical long axis of heart in such dogs. Mean VHS in VD or DV views has been reported to be significantly larger than that in lateral view. They also reported that the higher VHS in right lateral recumbency could be explained by the fact that divergence of X-ray beam and more distance of the heart from the cassette occurs in right lateral recumbency which leads to image magnification.

Bodh *et al.* (2016) reported that there were higher VHS values in right lateral recumbency than left lateral recumbency in Indian Spitz, Labrador retriever and Mongrel dogs.

Birks *et al.* (2017) reported that the median for right lateral VHS was significantly larger than left (10.3 versus 10.1) in Dachshund breed of dogs.

Puccinelli (2020) reported that there was non-significant differences in VHS were found between LL and RL thoracic radiographs of Chihuahuas. He determined that the possible variations in radiographic cardiac size during the cardiac cycle (diastolic vs systolic dimensions) also need to be taken into account. Further he explained that the Chihuahua breed does not show a significant sexual dimorphism and no significant differences in their thoracic morphotypes.

Taylor *et al.* (2020) reported non-significant effect of radiographic positioning (right or left recumbency) on VHS in Norwich Terriers.

2.2.5.1.1.7 Merits of the VHS

Buchanan and Bucheler (1995) reported that additional to initial assessment of heart size, the VHS method was useful in monitoring the progression of heart enlargement or recording changes in heart size in response to treatment over time in individuals because the short axis measurement includes right and left heart chambers, which increased with either right or left- sided heart enlargement. The VHS method was an useful aid to cardiac assessment in dogs, particularly for inexperienced observers who might especially be proned to false positive interpretations while examining radiographs of puppies, brachycephalic breeds or obese dogs because, these animals usually have a relatively broad, rounded cardiac silhouette that resembles the enlarged heart seen in many mesaticephalic or dolichocephalic dogs. They also reported that VHS reflects the overall dimension of the cardiac silhouette and incorporate all chambers of the heart, including the myocardium and the pericardium.

Buchanan (2000) and Hansson *et al.* (2005) reported that VHS was easy to perform and the measurements were relatively independent of both patient related and operator related variables.

Lamb *et al.* (2001) reported that VHS was accurate and beneficial in diseases that impose a volume load, such as mitral valve insufficiency, that result in eccentric hypertrophy or dilatation of the cardiac chambers, with a corresponding increase in the external dimensions of the heart. Further they added that, the observer's accuracy of diagnosis did not change significantly on using VHS as an adjunct to a subjective assessment of the radiographs.

Hansson *et al.* (2005) reported that VHS was unaffected by the experience of observer but does depend on the selection of the reference points of longitudinal and transverse axis of the heart and their conversion into VHS units. VHS method has the advantage of well-defined measurement points and objective numerical measurement.

Ljubica *et al.* (2007) opined that diagnosis of cardiac abnormalities in dogs could be based solely on VHS, and very suitable for clinical practice in their study on German shepherd dogs.

Marin *et al.* (2007) studied vertebral heart size in retired racing greyhounds and their study confirmed that the relative cardiomegaly reported in necropsy and echocardiographic studies in Greyhound was easily detected using plain radiography and the VHS. They further added that a high VHS suggest the presence of cardiac pathology, such as dilated cardiomyopathy, degenerative atrioventricular valvular disease, pericardial effusion, pericardioperitoneal diaphragmatic hernia, tricuspid dysplasia, ventricular septal defect and patent ductus arteriosus.

Woolley *et al.* (2007) revealed efficacy of pimobendan for treatment of MMVD was based on VHS.

Kraetschmer *et al.* (2008) reported that inspiration has non-significant effect on the VHS of Beagle dogs. Similar findings were reported by Zahabapour *et al.* (2016) in native breeds of Iran.

Guglielmini *et al*. (2009) reported that VHS has been found as the most accurate radiographic index for identifying dogs with pericardial effusion and to differentiate it from other cardiac diseases. They also revealed that VHS was fairly accurate for exclusion of a cough of cardiac origin in dogs with MVD.

Lord *et al.* (2011) concluded that VHS and particularly, the rate of change of VHS in units per month was useful measurements for detecting onset of CHF in Cavalier King Charles Spaniels with mitral valve regurgitation.

Jaspen-Grant *et al.* (2013) reported that the VHS values in lateral radiographs were unaffected by the depth or broadness of the chest of dogs which was in contrast to intercostal space method where such variation does occur.

Birks *et al.* (2017) reported that VHS can be reliably performed by observers with varying degrees of clinical experience.

Mourya *et al.* (2018) concluded that VHS was easy to apply in clinical practice for determining the heart size particularly for inexperienced observers.

2.2.5.1.1.8 Demerits of the VHS

Buchanan and Bucheler (1995) opined that VHS does not help to distinguish between diseases causing the right and left sided heart enlargement.

Buchanan (2000) reported that VHS relies only on two linear dimensions of heart not on the entire circumference; therefore subtle changes in the cardiac size may not be detected by VHS.

Lamb *et al*. (2001) reported that VHS was inaccurate for the diseases that impose a pressure load, such as aortic stenosis that tend to result in concentric hypertrophy, where in the thickening of myocardium encroaches the ventricular lumen without any marked change in the external dimensions.

Nakayama *et al.* (2001) opined that normal heart size does not always indicate the healthy heart. So, only determination of VHS was not sufficient for patients having heart disease symptoms.

Nakayama *et al.* (2001) and Hansson *et al.* (2005) reported that long axis measurement was more variable than the short axis measurement which could be due to difficulty in accurately determining the exact location of the apex of the heart due to skin folds, superimposed ribs, pleural effusion and/or the most cranial portions of the liver in dogs with severe cardiomegaly.

Lamb *et al*. (2001) and Lamb and Boswood (2002) opined that the accuracy of optimal VHS value for separation of cardiac from non-cardiac diseased dogs of each breed was relatively low (range, 58% to 83%).

Lamb and Boswood (2002) reported that measuring the cardiac silhouette does not aid in diagnosis of cardiac disease because there was considerable overlap in results from dogs with cardiac disease and normal dogs. This overlap occurs partly because of dogs with concentric hypertrophy and those examined in the early stages of their disease may not have any significant cardiac enlargement and partly because certain breeds have relatively large appearing hearts.

Hansson *et al.* (2005) reported that VHS method was unaffected by the experience of observer but does depend on the selection of the reference points of long and short axis of the heart and their conversion into VHS units.

Sanchez *et al.* (2012) opined that the presence of cardiogenic pulmonary oedema can significantly hinder the measurement of the VHS because of the associated perihilar increase in radio-opacity, potentially also obscuring the caudal venacava.

Jaspen-Grant *et al.* (2013) reported that thoracic vertebral anomalies such as butterfly vertebrae and hemi vertebrae in brachycephalic breed dogs and thoracic intervertebral disc disease increase the incidence of erroneously high assessments of VHS.

Gugjoo *et al.* (2013a) reported that anomalous vertebrae in the thoracic column were associated with a significant increase in VHS of the Bulldog and Boston terrier and also stated that thoracic depth to width ratio did not have a significant effect on VHS.

Birks *et al.* (2017) reported that VHS reliably detect the global heart enlargement, and published studies correlating the relationship between specific chamber enlargement to VHS in dogs were lacking.

Hoque *et al.* (2019) opined that even when using breed-specific normal VHS ranges, there was still significant overlap between normal dogs and dogs with cardiac disease.

2.2.5.1.1.9 VHS in cardiac patients

Lamb *et al.* (2001) reported that VHS was most accurate for the diagnosis of cardiac disease in Yorkshire terrier and the Cavalier King Charles Spaniel breeds affected by predominantly dilative forms of cardiac disease.

Nakayama *et al.* (2001) opined that the VHS was established to create a more objective way of diagnosing cardiomegaly via thoracic radiography.

Tilley and Smith (2001) reported the diagnostic accuracy of VHS for diagnosing cardiac disease in German shepherd breed of dogs as 75%.

Haggstrom *et al.* (2008) reported from a prospective study that increased VHS was factor associated with prediction of outcome in cases of heart failure secondary to mitral valve insufliciency.

Carlsson *et al.* (2009) conducted a study on size and shape of right heart chambers in small breed dogs having mitral valve regurgitation with enlarged right heart chambers and found VHS of 13.2 ± 1.1v in dogs with CHF.

Guglielmini *et al.* (2009) conducted a study to evaluate the usefulness of VHS in coughing dogs with chronic mitral valve insufficiency. They concluded that the dogs with cough of non-cardiac origin had significantly lower VHS as compared with those with cough of cardiac origin. Furthermore, VHS < 11.4v was fairly accurate to rule out cough of cardiac origin dogs with MVD.

Lord *et al.* (2011) reported that VHS increases considerably six to twelve months before the development of CHF in dogs.

Guglielmini *et al.* (2012) reported that VHS had highest diagnostic accuracy of radiographic indexes with a cut off VHS value of >11.9v in dogs with mild pericardial effusion.

Reynolds *et al.* (2012) opined that VHS has been shown to be a predictive variable for ensuring congestive heart failure.

Cote *et al.* (2013) studied VHS in 50 dogs with cardiac tamponade and reported the sensitivity and specificity of VHS > 10.7 for identification of dogs with cardiac tamponade attributed to pericardial effusion as 77.6% and 47.8% respectively.

Gugjoo *et al.* (2013b) found significant increase of VHS in DCM and pericardial effusion. They inferred that VHS could be effectively used to diagnose DCM in Labrador retriever dogs.

Tai and Hwang (2013) reported the average VHS in 40 clinically healthy dogs as 9.69 ± 0.69v, in non-cardiac associated chronic respiratory disorders as 10.32 ± 0.78v, in heartworm infestation without moderate to severely enlarged atrium as 10.44 ± 0.84v, mild to moderate chronic degenerative mitral diseases (CDMD) with enlarged left atrium and concomitant tricuspid regurgitation as 10.52 ± 1.01 v and in cases of CDMD with markedly enlarged left atrium and concomitant tricuspid regurgitation as 12.7 ± 1.69v. They noticed significantly elevated VHS in CDMD dogs from other groups.

Apetrei *et al*. (2014) reported in retrospective study on dogs that VHS provides additional information about clinical examination, and can also aid in monitoring cardiac disease that evolves with size and shape changes.

Bodh *et al.* (2014) reported increased VHS of 11.55 ± 0.28v and 11.24 ± 0.25v in right and left lateral recumbency in Labrador retriever dogs with occult DCM.

Torad and Hassan (2014) recorded VHS in dogs with microcardia as 9.4 ± 0.6v (9.2 to 10.1v) and in dogs with cardiomegaly as 10.5 ± 0.4v (10.2 to 10.9v) in German shepherd breed of dogs.

Fuentes (2015) opined that ascertaining VHS index provides an early clue to heart diseases like cardiomegaly.

Bodh *et al.* (2016) recorded the right lateral VHS of 13.10v and left lateral VHS of 12.80v in a Doberman pinscher breed of dog with generalized cardiomegaly and CHF.

Ingole *et al.* (2016) reported VHS in dilated cardiomyopathy affected dogs as 12.12 ± 0.17v and noticed significantly elevated VHS in DCM affected dogs.

Kim *et al.* (2017) reported that the possibility of death in dogs with a VHS > 10.5v was two-fold higher than that in dogs with a VHS of 10.5v in their retrospective study on degenerative mitral valve disease in 168 small-breed dogs.

2.2.5.2 Electrocardiography

Ettinger and Surter (1970) defined the electrocardiogram (ECG) as a graphic record of voltage produced by cardiac muscle cells during depolarization and repolarization plotted against time. Electrocardiography is the process of recording these electrical changes.

Hazen *et al.* (1991) opined that electrocardiogram was used to evaluate the presence of cardiac enlargement and was widely available and a cost effective diagnostic tool. The most robust application for electrocardiography was the evaluation of arrhythmias or conduction abnormalities.

Martin (2000) reported that increased S wave amplitude was associated with right ventricle enlargement commonly seen in heart failure of right sided origin.

Martin (2002) reported that a depression in ST segment of >0.2mV was associated with ventricular muscle abnormality, while increased duration of QRS complex indicates left ventricular cnlargement.

Dukes-McEwan *et al.* (2003) and Atkins *et al.* (2009) opined that ECG was not recommended as sole specific diagnostic tool neither in diagnosis of canine chronic valvuar disease nor in dilated cardiomyopathy in dogs.

Pereira *et al*. (2004) reported that increase in the amplitude of R wave, QRS duration and deep Q wave in Lead II ECG constituted a good indicator of cardiomyopathy in Cocker spaniel.

Coleman and Robson (2005) reported that electrocardiography was commonly performed on dogs in clinical practice and useful tool in diagnosing most cardiac arrhythmias and conduction disturbances, and in drawing inferences about cardiac chamber enlargement.

Abbott (2008) opined that electrocardiography was useful primarily for the diagnosis of arrhythmias, but was insensitive gauge of cardiac chamber size.

Martin *et al.* (2009) reported atrial fibrillation in 45 per cent, ventricular premature complexes in 31 per cent and supraventricular premature complexes in 9 per cent of dogs with dilated cardiomyopathy.

Kumar *et al.* (2010) conducted a retrospective study on hypertrophic cardiomyopathy in dogs and reported the electrocardiographic abnormalities like increased R wave amplitude, deep Q wave, ST coving, wide and bizarre QRS complexes.

Rasmussen *et al.* (2012) reported that in early stages of MMVD, sinus arrhythmia was often present, but during progression to CHF, tachycardia usually develops and the sinus arrhythmia ceases.

Savarino *et al.* (2012) reported that the diagnostic performance of P wave duration for identification of left atrial enlargement in dogs presents considerable limitations. The presence of left atrial enlargement was an important prognostic factor in several cardiovascular diseases and P wave duration should not be considered as a reliable indicator.

DeFrancesco (2013) reported that an ECG could further characterize the bradycardia into sinus bradycardia, a sinus node dysfunction, atrial standstill, or high-grade AV block, second-degree AV block or complete AV block.

Rajkumar (2013) reported that P wave in an electrocardiogram was not a sensitive indicator of left atrial enlargement.

Bodh *et al.* (2014) reported electrocardiogram was neither sensitive nor specific for the identification of dogs destined to develop DCM or those with asymptomatic disease. They observed increased QT interval (0.25 seconds) in Labrador retriever dogs with occult DCM and opined that this could be due to higher mean heart rate (102.18 ± 4.94 beats/minute) in these dogs.

Gugjoo *et al.* (2014a) provided the reference range of electrocardiographic parameters in normal Labrador retriever breed of dogs *viz.*, average heart rate (101.4 ± 1.53 beats per minute); P wave amplitude (0.21 ± 0.0009 mV); R amplitude (1.95 ± 0.007 mV); T wave amplitude (0.43 ± 0.008 mV); P wave duration (0.04 +0.00 seconds); P-R interval (0.099 ± 0.001 seconds): QT interval (0.21 ± 0.007 seconds) and QRS width (0.05 ± 0.001 seconds).

Mukherjee *et al*. (2015) reported that the electrocardiogram (ECG) was the voltage time graph of the electrical activities of the heart and widely used as a powerful non-invasive diagnostic tool for monitoring heart rate, cardiac rhythm, conduction integrity and electrical axis.

Baisan *et al.* (2016) reported significantly increased QRS complex duration in dilated cardiomyopathy group than chronic valvular disease group dogs.

Mukherjee *et al.* (2020) recorded normal electrocardiographic parameters in Labrador retriever breed of dogs *viz.*, average heart rate (132.13 ± 8.5 beats per minute); P wave amplitude (0.23 ± 0.02 mV); R amplitude (1.41 ± 0.11 mV); T wave amplitude (0.23 ± 0.014 mV); P wave duration (0.04 ± 0.01seconds); P-R interval (0.07 ± 0.01 seconds): QT interval (0.18 ± 0.01 seconds) and QRS width (0.04 ± 0.01 seconds).

2.2.5.3 Echocardiography

Calvert *et al.* (1982) reported that two-dimensional images in DCM affected Doberman pinscher dogs revealed dilated chambers, diminished mitral valve motion and marked hypo kinesis of ventricles.

End-systolic left ventricular dimensions, including end-systolic diameter, end-systolic volume and end-systolic volume indexed to body surface area (ESVI) were other conventional echocardiographic variables, which may be used to identify systolic myocardial dysfunction in dogs with MVD (Kittleson *et al.*, 1984 and Serres *et al.*, 2008).

Echocardiography was necessary for the accurate diagnosis, assessment, and prognosis of dogs with DCM (Calvert, 1986). M-mode echocardiography has proven very useful in recognizing left heart dilatation and poor contractile function in affected dogs (Lombard, 1984).

Ejection Fraction (EF%) represents the per cent of blood volume ejected from the LV during systole. It is therefore defined by the per cent of change in LV volumes between the diastolic and systolic phases (EF% = (EDV-ESV) * 100/EDV, where EDV and ESV are the LV systolic and diastolic volumes), and a low EF% value is consistent with decreased systolic function (Boon, 1998 and Serres *et al.*, 2008).

Fractional Shortening per cent (which corresponds to a 1-dimensional assessment of myocardial systolic function) is defined by the per cent change in radial LV diameters between the diastolic and systolic phases (FS% = (LVIDd-LVIDs) * 100/LVIDd, where LVIDd and LIVDs are the LV systolic and diastolic diameters, usually assessed by M-mode echocardiography) and again a low FS per cent value is consistent with decreased contractility (Boon, 1998 and Serres *et al.*, 2008).

Schober *et al.* (1999) reported that in dogs with chronic MVD, cardiac contractility decreased slowly, but progressively and inexorably.

Rishniw and Erb (2000) reported that left atrial to aortic root ratio (LA:Ao) determined from the right parasternal short axis view was the most commonly used method for detecting left atrial enlargement in veterinary clinical practice.

Hansson *et al.* (2002) estimated left atrial to aortic root indices using two-dimensional (2-D) and M-mode echocardiography in Cavalier King Charles Spaniels with and without left atrial enlargement. They reported that 2-D index was more sensitive to LA enlargement than the M-mode index.

Chronic and hemodynamically significant mitral regurgitation resulted in volume overload, which was first characterized by left atrial enlargement as assessed by the LA/ Ao ratio (Chetboul *et al.* 2004 and Chetboul *et al.,* 2005).

Pyle *et al.* (2004) reported the cardiac sequelae (cor pulmonale) paralleled the severity of the pulmonary hypertension and the typical echocardiographic findings in severely affected dogs included an enlarged right ventricle, flattened inter ventricular septum, tricuspid regurgitation and a small left ventricle.

Kibar and Alkan (2005) opined that echocardiography was useful to detect intracardial disorders to evaluate the cardiac chamber size, wall thickness, wall motion, valve configuration and motion, and the great vessels.

Serres *et al.* (2008) reported that M-mode echocardiogram could be a valuable non- invasive method for gaining quantitative insight into systolic and diastolic cardiac function in dogs with DCM. They also opined that standard transthoracic echocardiographic examination was currently considered as the noninvasive diagnostic method of choice for early detection of the mitral valve lesions, evaluation of mitral regurgitation severity and lastly, for assessing its impact on cardiac remodeling, myocardial function, left ventricular (LV) filling pressures as well as pulmonary arterial pressure.

Borgarelli *et al.* (2008) studied a large population of dogs with MVD (n=558) and reported left atrium / aorta (LA/Ao) >1.7 was the only variable significantly associated with survival time when cardiac-related death was considered.

Bonagura and Schober (2009) reported that EF% and FS% were the two indices most commonly used to assess systolic myocardial function in the dog by conventional echocardiography.

Bodh *et al.* (2014) reported increased left ventricular mass, increase in diastolic and systolic left ventricular internal dimensions, reduced fractional shortening and ejection fraction, increased left atrial diameter and LA/ Ao ratio in Labrador retriever dogs with occult DCM.

Gugjoo *et al.* (2014b) provided the reference range of echocardiographic parameters in normal Labrador retriever breed of dogs viz., LVIDd (mm) 37.58 ± 1.05 (29.4-45.3), LVIDs (mm) 23.98 ± 0.97 (14.5-36.8), IVSd (mm) 9.06 ± 0.37 (5.6-13.5), IVSs (mm) 14.47 ± 0.64 (8.1-20.8), LVPWd (mm) 8.75 ± 0.26 (6.2-11.3), LVPWs (mm) 12.08 ± 0.4 (9.1-14.7), EDV (ml) 65.42 ± 3.58 (26.97-93.82), ESV (ml) 22.38 ± 2.14 (5.57-57.35) and EF (%)65.50 ± 2.15 (45.45-81.35).

Cubas *et al.* (2017) reported that if the size of right ventricle was more than one third of left ventricle, it should be considered as right ventricular enlargement in a retrospective study on tricuspid valve dysplasia in dogs.

Chetboul *et al.* (2018) reported in prospective study on French Bulldogs that the right ventricular size was considered as normal when the ratio between the right ventricular internal diameter and the left ventricular internal diameter in diastole was ≤0.37 and the right atrial size was considered as normal if the right atrium-to-left atrium ratio was ≤1 in the right parasternal long axis 4 chamber view during echocardiogram.

Keene *et al.* (2019) reported that the left ventricular size was considered as normal when the left ventricular internal diameter in diastole was <1.7 and left atrial size was considered normal when the left atrium-to-aorta ratio was <1.6 during echocardiogram.

2.2.5.4 Comparison of VHS with electrocardiography and echocardiography

Buchanan and Bucheler (1995) noticed that absolute M mode echocardiographic measurements in dogs with progressive cardiomegaly over a period of 2.5 years did not reflect the extent of heart size increase as good as the VHS method. The echocardiographic measurements represented only single dimension *i.e.,* mainly short axis, whereas the VHS method reflected change in two dimensions. Therefore, they concluded that VHS was superior to echocardiography in objectively measuring the progression of heart disease.

Nakayama *et al.* (2001), Hansson *et al.* (2005), Gulanber *et al.* (2005), Bavegems *et al.* (2005), Marins *et al.* (2007) and Gugjoo *et al.* (2013a) confirmed that the VHS correlates well with echocardiographic values.

Nakayama *et al.* (2001) considered VHS as gold standard for diagnosing cardiomegaly, and noticed a strong correlation of VHS value with P wave duration and QRS duration of ECG and left atrium-to-aorta ratio, left ventricular end-diastolic diameter, left ventricular end-systolic diameter of echocardiography in 16 dogs with cardiomegaly induced by rapid ventricular pacing.

Sisson (2002) cited that electrocardiograms are frequently normal in dogs with mitral regurgitation even when cardiomegaly can be demonstrated on radiographs or on echocardiogram.

Guglielmini (2003) reported that radiography was useful for evidencing signs of right ventricular enlargement. Echocardiography gives the evidence of both left sided and right sided cardiac hypertrophy.

Kibar and Alkan (2005) reported that for the reliable diagnosis of the heart diseases, radiological, echocardiographic and colour Doppler examinations must be performed systematically.

Fine (2008) suggested that DCM could be typically diagnosed using a combination of radiographs, ECG and echocardiogram.

Saida *et al.* (2008) reported a significant positive relationship between VHS and echocardiographic parameters like LA/Ao, peak E wave velocity, peak A wave velocity, cardiac index, left ventricular end diastolic volume index, stroke volume index and heart rate from a comparative study of relationships between VHS and echocardiographic parameters in 34 dogs suffering from mitral regurgitation.

Bavegems *et al.* (2009) reported a significant correlation between the R wave amplitude in lead II and the VHS from left lateral and right lateral thoracic radiographs and the left ventricular internal diameter in systole and diastolic from echocardiography.

Mato (2012) and Braslasu *et al.* (2013) stated that ECG was primarily used to detect arrhythmias and measure heart rate, however ECG was less sensitive for assessing the size of the heart. Thoracic radiography can help in differentiating dyspnoea and causes of cough *i.e.,* to distinguish between primary respiratory disease and heart problem, however, less sensitive to heart size. Echocardiography can assess the size of the heart, myocardial function, heart valves as well as the direction velocity and character of the blood flow.

Pace (2016) opined that electrocardiogram (non-sinus arrhythmia or ventricular premature complexes), VHS (>10.7v) or cardiac biomarkers (natriuretic peptide >900pmol/L) was considered in early detection protocol for heart diseases diagnosis in dogs, if echocardiography facility was not available.

Bodh *et al.* (2016) concluded that radiographic, electrocardiographic and echocardiographic findings helped in diagnosis of dilated cardiomyopathy and pericardial effusion in dogs.

Birks *et al.* (2017) reported non-significant correlation between left atrial echocardiographic parameters and VHS in a study on 51 Dachshund dogs.

Rajamohan (2018) reported electrocardiography showed atrial fibrillation, in radiograph, cardiomegaly was noticed and vertebral heart score of 12 was measured. Echocardiography revealed an increased LVIDd of 5.4cm, LVIDs of 4.8cm, reduced FS of 10 per cent in M-mode. On 2-D echocardiography, dilated left atrium and increased LA/AO ratio (left atrium / aorta) was recorded and an EF of 38.5% in nine year old male

Labrador retriever dog and reported that electrocardiography, radiography and echocardiographic values were in accordance with the earlier reports.

Hoque *et al*. (2019) reported that there was a fair correlation between VHS measurements and a variety of other indices of cardiac chamber enlargement, including end-systolic and end-diastolic ventricular diameters as well as duration of the P wave and QRS complex.

Materials and Methods

III. MATERIALS AND METHODS

3.1 Design of the study

The study was carried out among the Labrador retriever dogs presented to Veterinary College Hospital, Hebbal, Bengaluru. The clinically normal Labrador retriever dogs and Labrador retriever dogs with established cardiac disorders were selected based on the history, clinical examination, radiography, electrocardiography and echocardiography findings.

Twelve dogs of either gender were selected for study and all the twelve dogs were subjected for clinical, physiological, hematological, biochemical, radiography, electrocardiography and echocardiography and they were divided into two groups *viz.*, Group A and Group B.

Group A: Six normal Labrador retriever dogs of either gender were subjected for VHS evaluation on both right and left lateral recumbency.

Group B: Six Labrador retriever dogs of either gender with established cardiac disorders were subjected for VHS evaluation on both right and left lateral recumbency.

3.2 Instrumentation

3.2.1 X-Ray Unit

Allengers HF with 110 KVp unit capacity portable X-ray machine was used to obtain radiographs in both right and left lateral recumbency. Carestream X-ray films were utilized for radiography **(Plate 01).**

3.2.2 Electrocardiographic equipment

MAC 400 (Wipro GE Health care Pvt. Ltd, Bengaluru) 3 channel, 12 lead electrocardiograph was used to record the electrocardiogram **(Plate 02).** The electrocardiograph recording paper (Morquitte Hellige Medical Systems), a thermosensitive paper of a recording width of 75mm was used. An electrically conductive gel (Meditech® Kardia cares, Chennai) was used as a conducting medium for application of electrodes commercially available as crocodile clips used for connecting electrical circuits were modified and used to connect the electrodes of electrocardiograph to skin.

3.2.3 Echocardiography machine

Logiq Book XP from general electronics was used for echocardiography. A microconvex transducer of 6-10 MHz capacity was used **(Plate 03).** A table with two openings was used to scan the dogs from dependant side **(Plate 04).** A coupling gel (Meditech® Kardia cares, Chennai) was used to improve the contact between the transducer and the dog and to improve the image quality.

3.2.4 Blood pressure monitor

Suntech® automatic blood pressure monitor was utilized to measure the blood pressure before and after the VHS evaluation in the dogs **(Plate 05).**

3.3 Occurrence

The occurrence of cardiac disorders irrespective of age, breed and sex of dogs was recorded for a period of 12 months from June 2017 to May 2018. Along with that the

Plate 01: Allengers HF with 110 KVp unit capacity portable X-ray machine

Plate 02: GE's MAC 400 (Wipro GE Health care Pvt. Ltd, Bengaluru) 3 channel, 12 lead electrocardiograph

Plate 03: Logiq Book XP from General Electronics with microconvex transducer of 6 to 10 MHz capacity

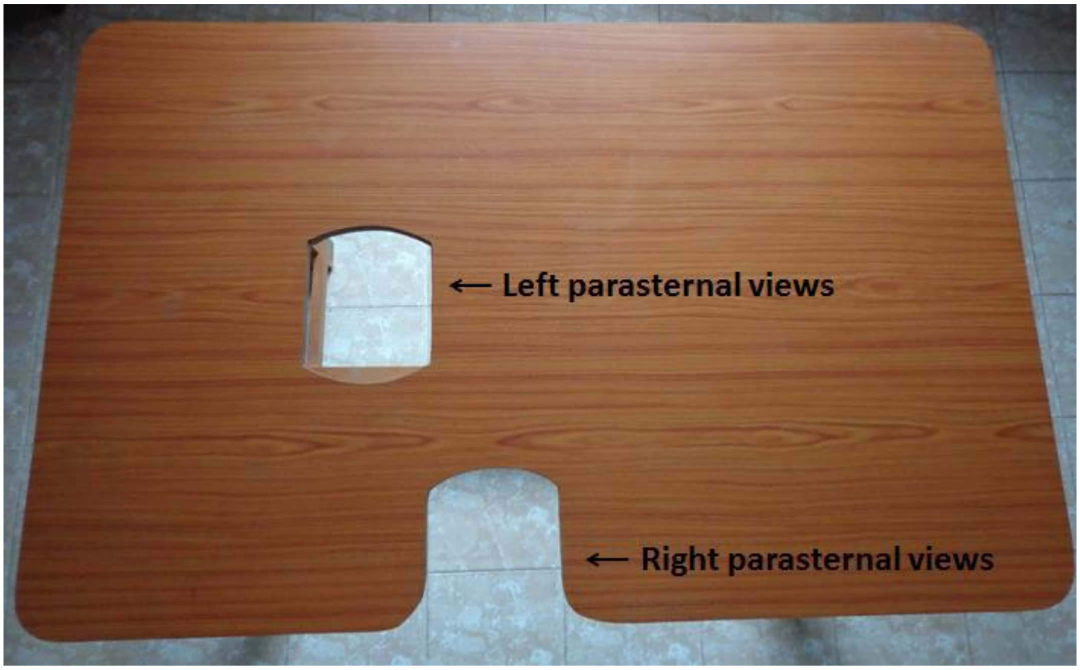


Plate 04: Echocardiographic examination table with two openings

Plate 05: Suntech® automatic blood pressure monitor

occurrence of cardiac disorders in Labrador retriever breed of dogs was recorded with regard age and sex.

3.4 Parameters studied

3.4.1 Clinical parameters

Body Condition Score (BCS) of all the dogs was recorded as per the 9-point scale prescribed by the Nestle Purina body condition system (Laflamme DP. Development and Validation of a Body Condition Score System for Dogs. Canine Practice July/August 1997; 22:10-15) **(Plate 06).**

3.4.2 Physiological parameters

Rectal temperature (^{o}F), Heart rate (beats/min), Respiratory rate (breaths/min) and Blood pressure (mmHg) were recorded before and after VHS evaluation in all the dogs under research study.

3.4.3 Hematological parameters

Packed cell volume (PCV) (%), Total erythrocyte count (TEC) (millions/cmm), Hemoglobin (g/dL), Total leukocyte count (TLC) (thousands/cmm) and Differential leukocyte count (DLC) (%) were estimated by standard methods (Benjamin, 1998) by collecting 2 ml of blood from cephalic vein in EDTA vial before and after VHS evaluation in all the dogs under research study. All the above mentioned haematological parameters were analysed on the same day of collection.

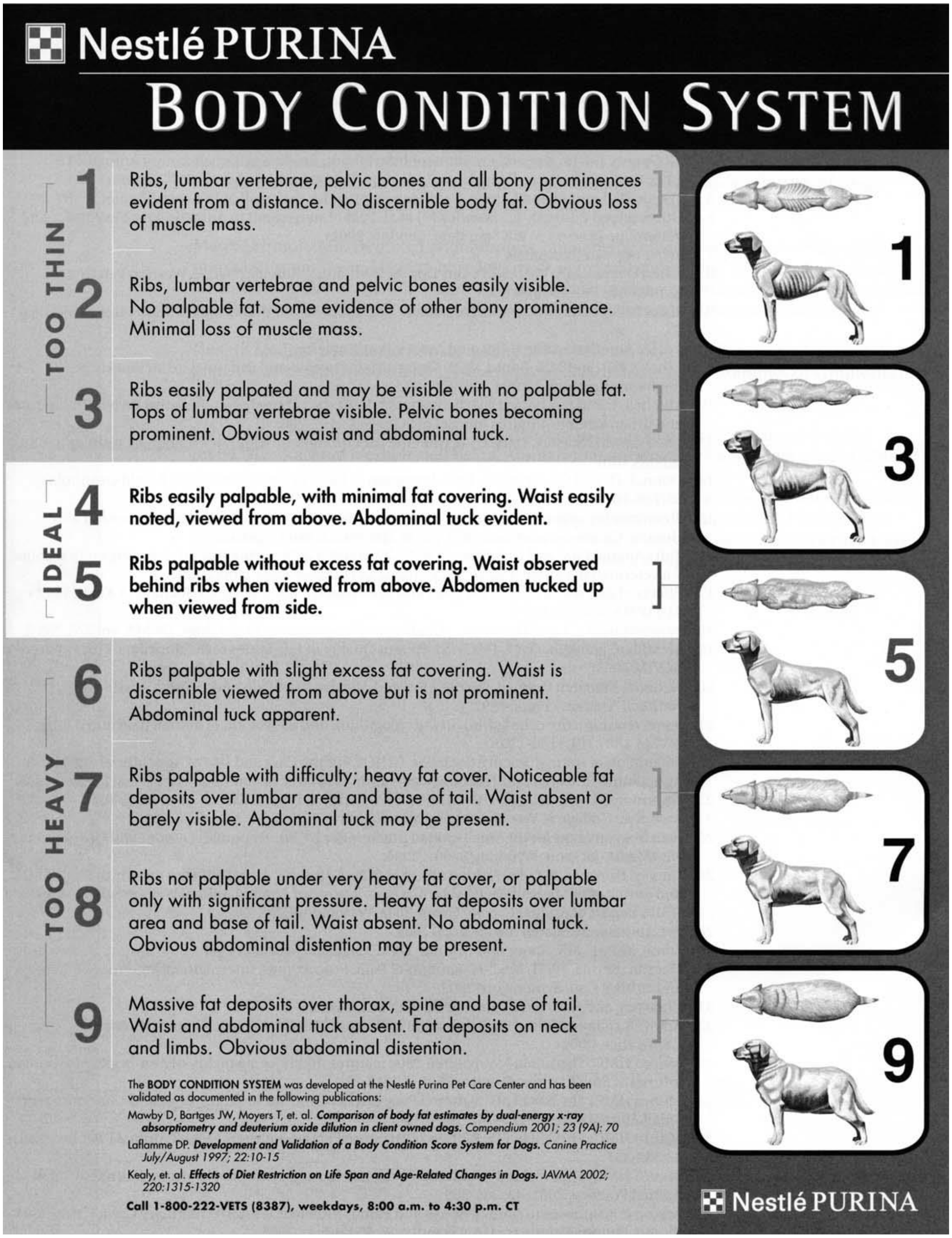


Plate 06: Nestle Purina Body Condition Scoring System.

3.4.4 Biochemical parameters

Blood samples were collected and serum was separated before and after VHS evaluation for estimation of Serum creatinine (mg/dL), Alanine amino transferase (ALT) (IU/L) and Blood urea nitrogen (BUN) (mg/dL) by standard method using ARTOS biochemical analyser (M/s. Swemed Diagnostic, Bengaluru) using respective diagnostic kits as per the manufacturer's instruction.

3.4.5 Diagnostic parameters

3.4.5.1 Thoracic radiography

Radiographs were taken in conscious dogs without sedation or anesthesia using standard exposure techniques. The thorax of the dog was positioned as close to the film as possible, to include all the thoracic vertebrae in radiographs. The subjective parameters of radiographic abnormalities of heart and thorax were recorded and viewed using standard pattern given by Berry *et al.* (2009).

For right lateral thoracic radiograph, the dogs were placed on right lateral recumbency (right down) and X-ray beam enters from left side of thorax. The radiograph was marked as right lateral. The forelimbs were pulled cranially and the head and neck extended slightly, the central axis of X-ray beam should be centered just caudal to the caudal aspect of left scapula **(Plate 07).** For left lateral thoracic radiograph, the dogs were placed on left lateral recumbency (left down) and X-ray beam enters from right side of thorax. The radiograph was marked as left lateral. The forelimbs were pulled cranially and the head and neck extended slightly, the central axis of X-ray beam should be centered just caudal to the caudal aspect of right scapula **(Plate 08).**

Plate 07: Right lateral positioning of dog for right lateral thoracic radiograph

Plate 08: Left lateral positioning of dog for left lateral thoracic radiograph.

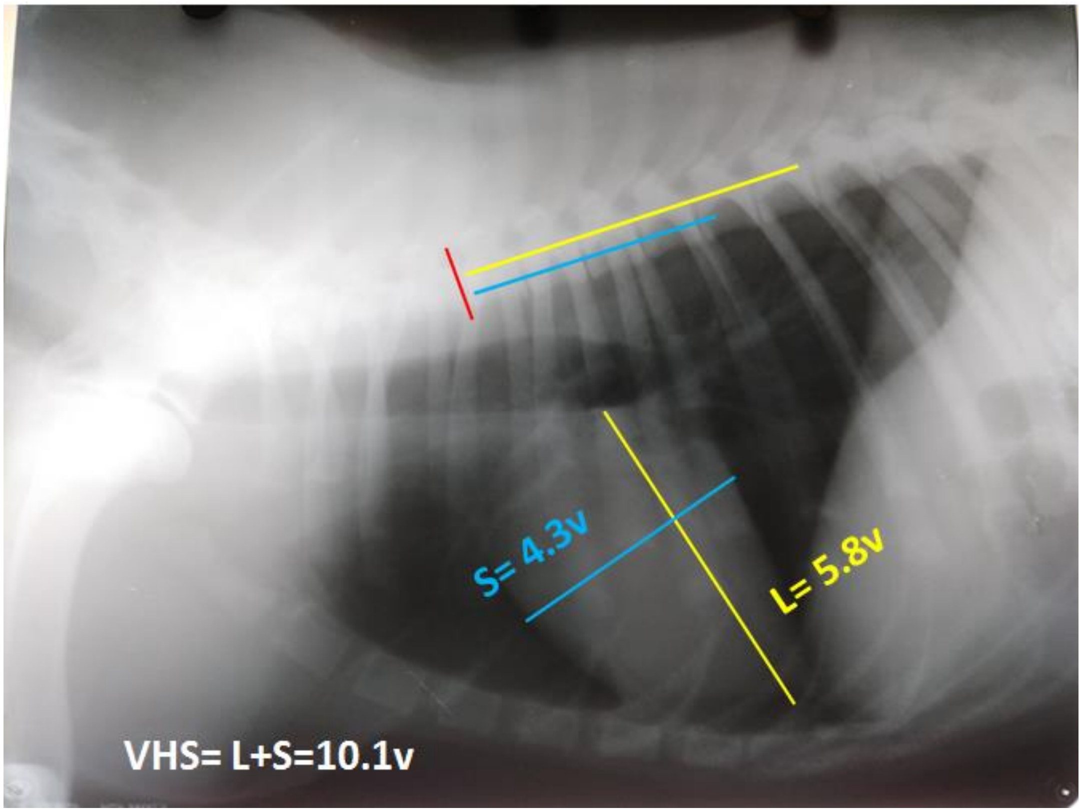
3.4.5.1.1 Vertebral Heart Score (VHS) parameters

Long axis (L), Short axis (S) and VHS values were measured in both right and left thoracic radiographs as per the standard procedure using the formula, VHS= L+S as reported by Buchanan and Bucheler (1995). Long axis was measured from ventral border left main stem bronchus to the cardiac apex using a measuring scale and short axis was taken from central third region of heart perpendicular to the long axis with a measuring scale on both lateral thoracic radiographs. The sum of these measurements were then compared to the mid-thoracic vertebral bodies starting from the anterior edge of the 4^{th} thoracic vertebra (T4). Summation of number of vertebrae in relation to long and short axis of heart indicates Vertebral Heart Score (VHS) **(Plate 9 and 10).**

These recorded VHS values of Labrador retriever dogs were compared to evaluate the effect of right and left lateral recumbency on VHS values in the Labrador retriever breed of dog.

3.4.5.2 Electrocardiography (ECG)

The ECG was recorded with conscious dogs in right lateral recumbency on a non-conductive surface with the leads being connected proximal to olecranon on the caudal aspect of the appropriate forelimb and over patellar ligament on the cranial aspect of the appropriate hind limb. 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 jaws slightly bent apart to avoid pinching of the skin at the site of electrode application, this prevented the muscle contraction and subsequent artefacts in ECG **(Plate 11).**



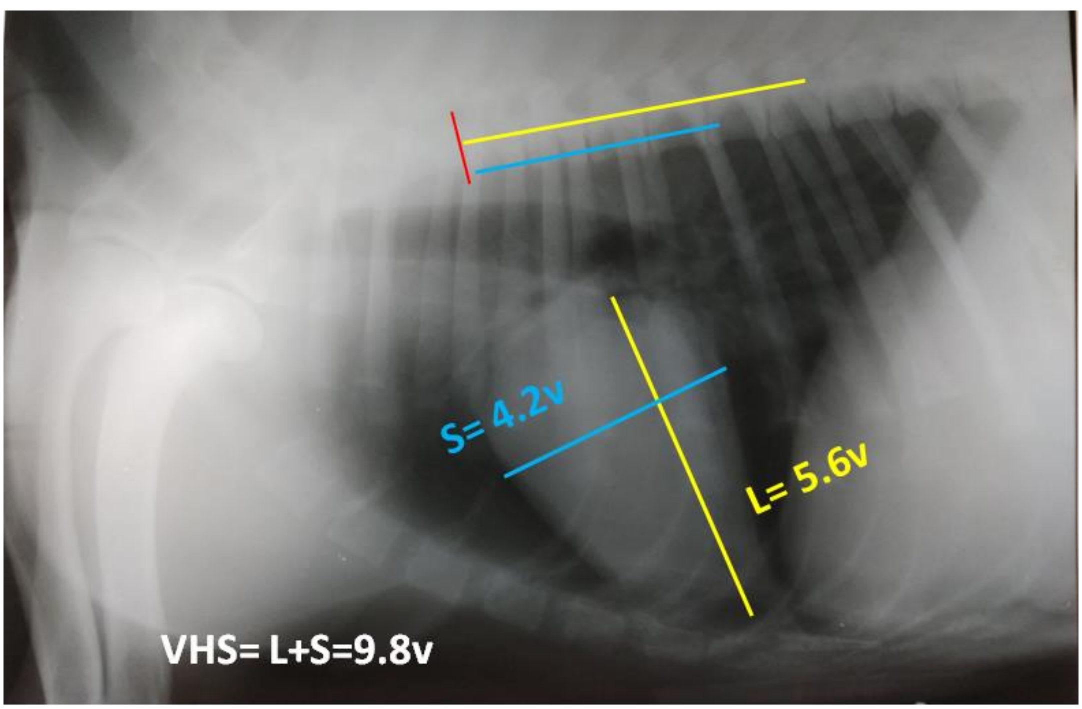


Plate 09 & 10: Right and left lateral thoracic radiographs of dog1 (Group A) showing measurements of long axis (L), short axis (S) and Vertebral Heart Score (VHS).

Plate 11: Right lateral recumbent positioning of the dog for electrocardiography

Plate 12: Positioning of the dog for echocardiography (right parasternal view)

The ECG was recorded at 25 mm/s paper speed and 10 mm=1 mV calibration on a thermosensitive paper of a recording width of 75mm. Each small square on horizontal axis represents 0.04 seconds and on vertical axis represents 0.1 millivolt (mV). Amplitude and duration of P, Q, R, S, and T waves were measured together with PR interval, QRS interval, RR interval and QT interval in lead II as its electrode direction matched the typical waves as the depolarization vector.

3.4.5.3 Echocardiography

The echocardiographic examination was performed in conscious non sedated dogs. The cardiac area was trimmed and shaved before examination. The cardiac area was made overlying on fenestration on echocardiographic table to permit scanning from dependant side using microconvex transducer of 6-10MHz **(Plate 12)**. This technique was used as supplementary tool to the present study.

3.4.5.4 Correlation of VHS findings with ECG and Echocardiography

The obtained VHS values of Group A and Group B dogs were subjectively correlated with the reference values of ECG and subjective parameters of two-dimensional and M mode echocardiography for Labrador retriever breed of dogs.

3.5 Statistical analysis

The mean and standard error of all the parameters were calculated as per Snedeecor and Cochran (1994). The clinical, physiological, haematological, biochemical and diagnostic parameters within the group and between the groups were calculated using T test: paired two sample for means and T test: two sample assuming equal variences respectively as described by Snedeecor and Cochran (1994).

Results

IV. RESULTS

The present study was undertaken in six normal Labrador retriever dogs and six Labrador retriever dogs with established cardiac disorders of either gender presented to Veterinary College Hospital, KVAFSU, Hebbal, Bengaluru and the results of the study were presented as follows.

4.1 Occurrence of cardiac disorders in dogs

A total of 8472 dogs were presented to the Veterinary College Hospital, KVAFSU, Hebbal, Bengaluru during June 2017 to May 2018. Among these, 165 (1.94%) cases were diagnosed to be affected with cardiac diseases **(Fig. 1).**

In breed-wise occurrence of cardiac diseases **(Table 1, Fig. 2),** the highest was in Labrador Retriever *i.e.,* 24.84% (41/165) followed by Mongrel (14.54%; 24/165), Pomeranian (11.53%; 19/165), Golden Retriever (9.7%; 16/165), Pug (9.09%; 15/165), German Shepherd (6.67%; 11/165), Boxer (6.07%; 10/165), Great Dane (3.63%; 6/165), Rottweiler (3.03%; 5/165) and other dog breeds (Siberian Husky, Saint Bernard, Dachshund and Doberman) constituted 10.9% (18/165).

In age-wise occurrence of cardiac diseases **(Table 2, Fig 3),** 43.63% (72/165) were middle age dogs ranging from four to eight years followed by old age dogs of more than eight years of age *i.e.,* 35.15% (58/165) and the least occurrence was seen in young age group of zero to four years *i.e.,* 21.21% (35/165).

In gender-wise occurrence of cardiac diseases **(Table 3, Fig 4),** among 165 dogs with cardiac diseases, 66.66% (n=110) were males and 33.33% (n=55) were female dogs.

Table 1: Breed-wise occurrence of cardiac diseases

Breed	No. of animals affected	% of occurrence
Labrador Retriever	**41**	**24.84**
Mongrel	24	14.54
Pomeranian	19	11.53
Others	18	10.9
Golden Retriever	16	9.7
Pug	15	9.09
German Shepherd	11	6.67
Boxer	10	6.07
Great Dane	6	3.63
Rottweiler	5	3.03

Fig. 1: Pie diagram showing the occurrence of cardiac disorders in dogs

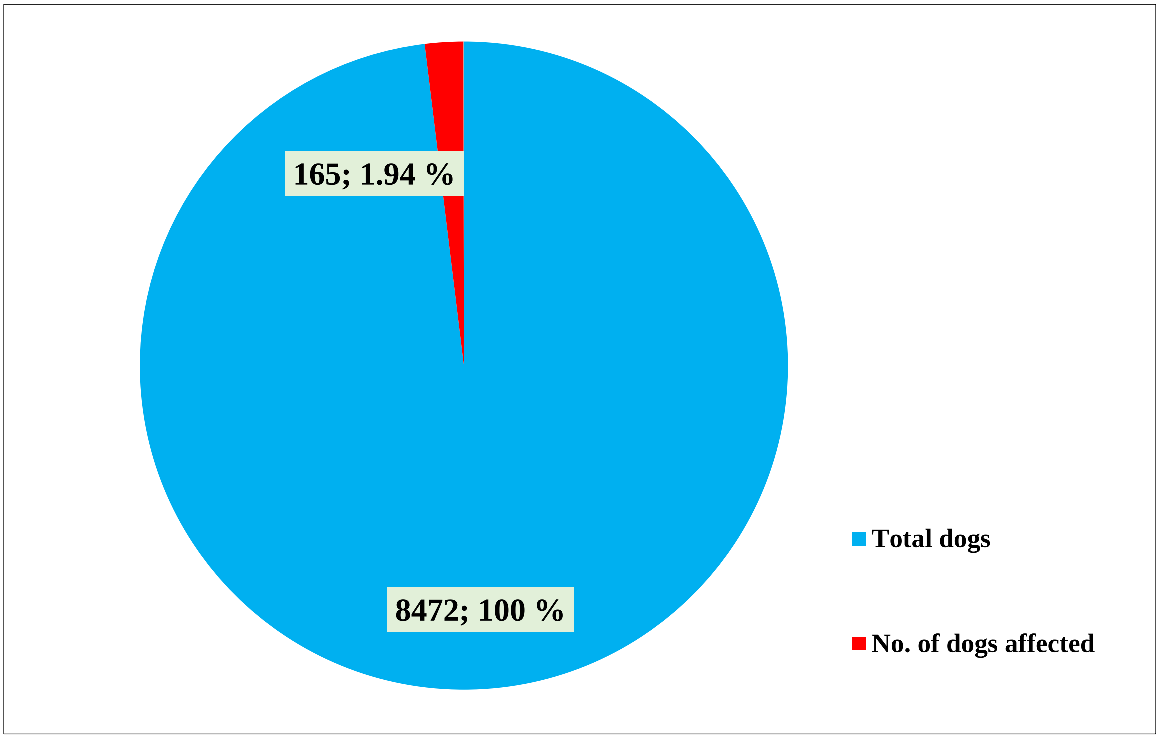


Fig. 2: Pie diagram showing the breed-wise occurrence of cardiac diseases in dogs

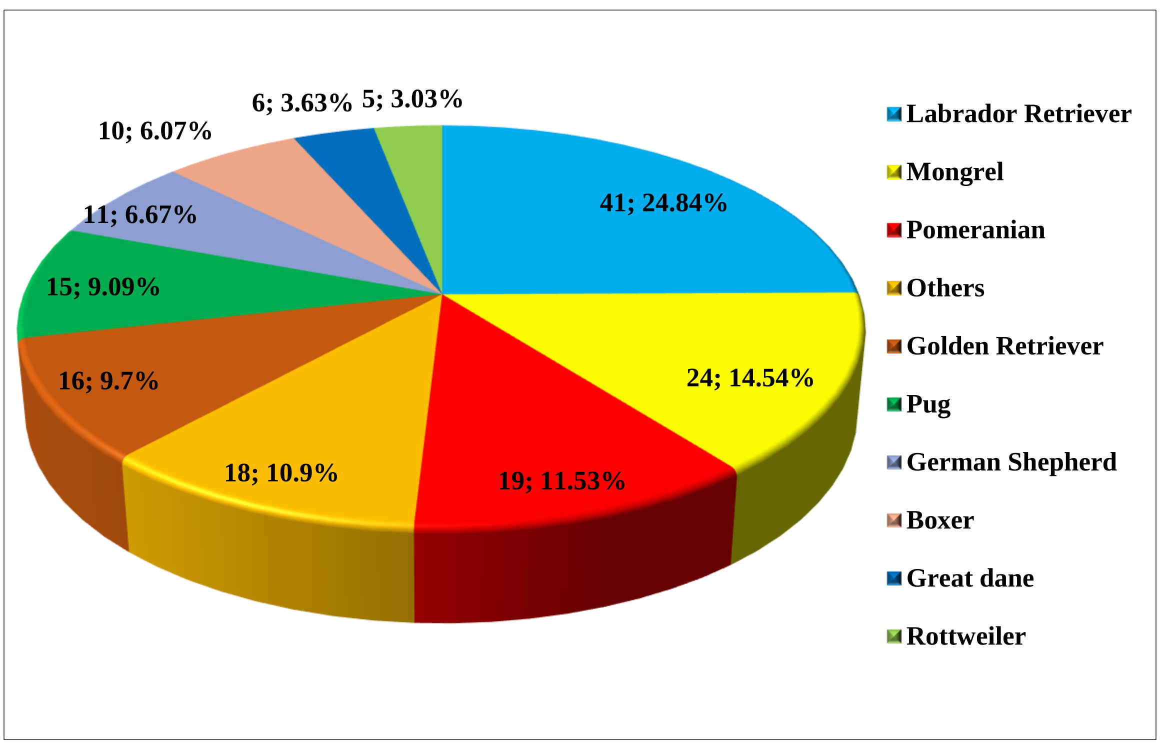


Table 2: Age-wise occurrence of cardiac diseases

Age group	No. of animals affected	% of occurrence
0-4 years	35	21.21
4-8 years	72	43.63
>8 years	58	35.15

Table 3: Gender-wise occurrence of cardiac diseases

Gender	No. of animals affected	% of occurrence
Male	110	66.66
Female	55	33.33

Fig. 3: Pie diagram showing the age-wise occurrence of cardiac diseases in dogs

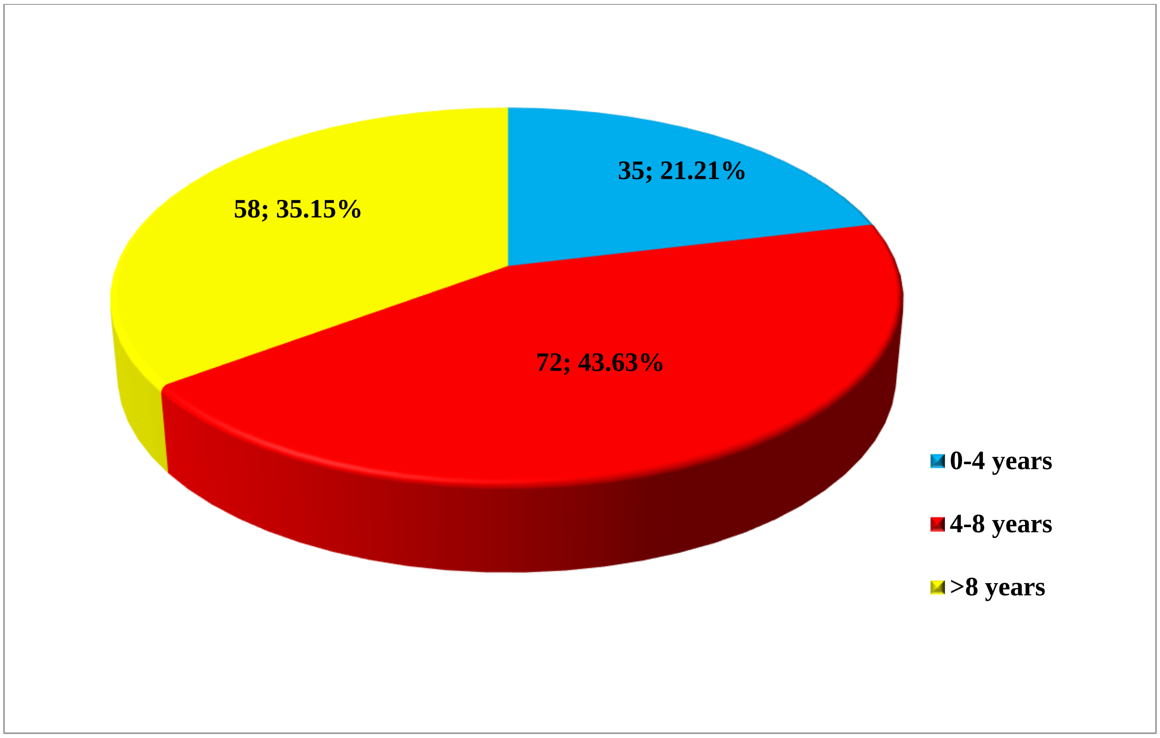
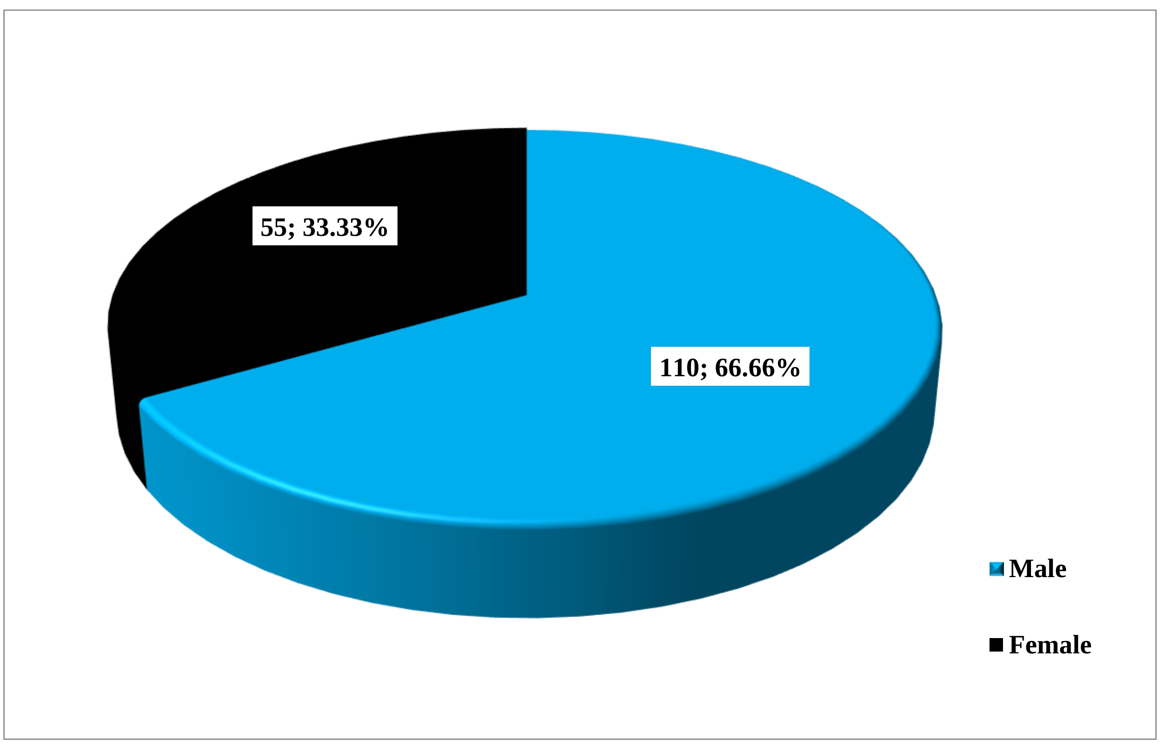


Fig. 4: Pie diagram showing the gender-wise occurrence of cardiac diseases in dogs



4.2 Grouping of animals

Twelve Labrador retriever dogs of either gender were selected for study and they were sub divided into two groups *viz.*, Group A and Group B **(Table 4).**

In Group A: Among six Labrador retriever dogs selected for the study, four were males and two were females with normal clinical, physiological, hemato-biochemical, radiography, electrocardigraphy and echocardiographic parameters for VHS evaluation on both right and left lateral recumbency. The results were recorded as follows.

In Group B: Among Six Labrador retriever dogs selected for the study, four were males and two were females with established cardiac disorders *viz.*, right ventricular enlargement (3), bilateral ventricular enlargement (1), right atrial enlargement (1) left atrio-ventricular enlargement (1), among which one dog showed thickening of mitral valve and regurgitation of the same indicative of Myxomatous Mitral Valve Disease (MMVD) using the same parameters as in group A and the results were recorded as follows.

Table 4: Grouping of the animals with Mean ± SE values of physical parameters

Parameters		Group A	Group B	P value
Age (in years)		6.5 ± 0.9 (3.5 to 9)	7.6 ± 0.65 (5.5 to 10)	0.32
Body weight (in kg)		29.38 ± 1.03 (25.3 to 32)	33.63 ± 0.36 (32 to 34.5)	0.03
Gender	Male	04	04	……
	Female	02	02	

4.2.1 Age

The Mean ± SE age of Group A dogs was 6.5 ± 0.9 years, which ranged between 3.5 to 9 years and Group B dogs was 7.6 ± 0.65 years, which ranged between 5.5 to 10 years. Within the Group B dogs, the highest occurrence of cardiac diseases was seen in 5 to 8 years age followed by the dogs with more than 8 years **(Table 5).** The mean values of the age between the Group A and Group B dogs were statistically not significant (P value $\geq$ 0.05).

Table 5: Age-wise occurrence of cardiac diseases within the Group B dogs

Age group	No. of animals affected	% of occurrence
5-8 years	04	66.66
>8 years	02	33.33

4.2.2 Gender

In both Group A and Group B, gender wise were equally represented by four male dogs and two female dogs. In Group B dogs, the occurrence of cardiac diseases was more in males (66.66%, 4/6) as compared to females (33.33%, 2/6).

4.2.3 Body weight

The Mean ± SE value of body weight was 29.38 ± 1.03 kg in Group A dogs, ranged between 25.3 to 32 kg whereas in Group B dogs it was 33.63 ± 0.36 kg, which ranged between 32 to 34.5 kg. The variations in mean values of the body weight between the groups were statistically significant (P value $\leq$ 0.05).

4.3 Diagnosis of cardiac diseases

4.3.1 Clinical signs and physical examination

All the dogs in the Group B showed one or more signs suggestive of cardiac diseases such as exercise intolerance, weakness, pale mucous membrane, cough, tachypnoea, ascites, tachycardia, inappetance and elevated body temperature **(Table 6)**. Two dogs showed severe dry coughing, which increased on exercise and were not responding to medical therapy.

Table 6: Clinical signs and physical examination findings in the Group B dogs

Clinical signs	B 1	B 2	B 3	B 4	B 5	B 6
Exercise intolerance	+	+	+	+	+	+
Weakness	+	-	+	-	+	+
Syncope	-	-	-	-	+	-
Cough	+	+	+	+	++	++
Pale mucous membrane	-	-	+	-	-	+
Tachycardia	+	+	+	+	+	+
Tachypnoea	-	+	-	+	+	+
Ascites	+	-	+	-	+	-
Elevated body temperature	-	-	-	+	+	-
Inappetance	+	+	-	-	+	-

"+" Presence of clinical sign, "-" Absence of clinical sign and "++" Severe cough

4.3.2 Clinical parameters

4.3.2.1 Body Condition Score (BCS)

The Mean ± SE of BCS value was 6.03 ± 0.18 in Group A dogs, which varied between 5.5 to 6.5 and that of Group B dogs was 6.5 ± 0.13, which varied between 6.2 to

7.1. The variation in the mean values of the BCS between the Group A and Group B dogs was statistically non-significant (P value ≥ 0.05) **(Table 7 and Fig. 5)**.

Table 7: Mean ± SE values of BCS in both Group A and B dogs

Clinical parameter	Group A	Group B	P value
Body Condition Score (BCS)	6.03 ± 0.18	6.5 ± 0.13	0.06

4.3.3 Physiological parameters

The Mean ± SE values of various physiological parameters in both Group A & B dogs before and after VHS evaluation was depicted in **Table 8.**

4.3.3.1 Rectal temperature (ºF)

The Mean ± SE values of rectal temperature in Group A dogs before VHS evaluation was 101.8 ± 0.16 ºF, which ranged between 101.3ºF to 102.4 ºF and the same after VHS evaluation was 101.9 ± 0.14 ºF, which ranged between 101.5ºF to 102.3 ºF.

The Mean ± SE values of rectal temperature in Group B dogs before VHS evaluation was 101.9 ± 0.51ºF, which ranged between 100.1ºF to 103.4 ºF and the same after VHS evaluation was 101.8 ± 0.75ºF, which ranged between 98.3ºF to 103.5 ºF.

The variations in the rectal temperature were within normal reference range in Group A dogs and statistically non-significant both within and between the groups (P value ≥ 0.05) **(Fig.6).**

Fig. 5: Column showing the Mean ± SE values of body condition score in both Group A and B dogs

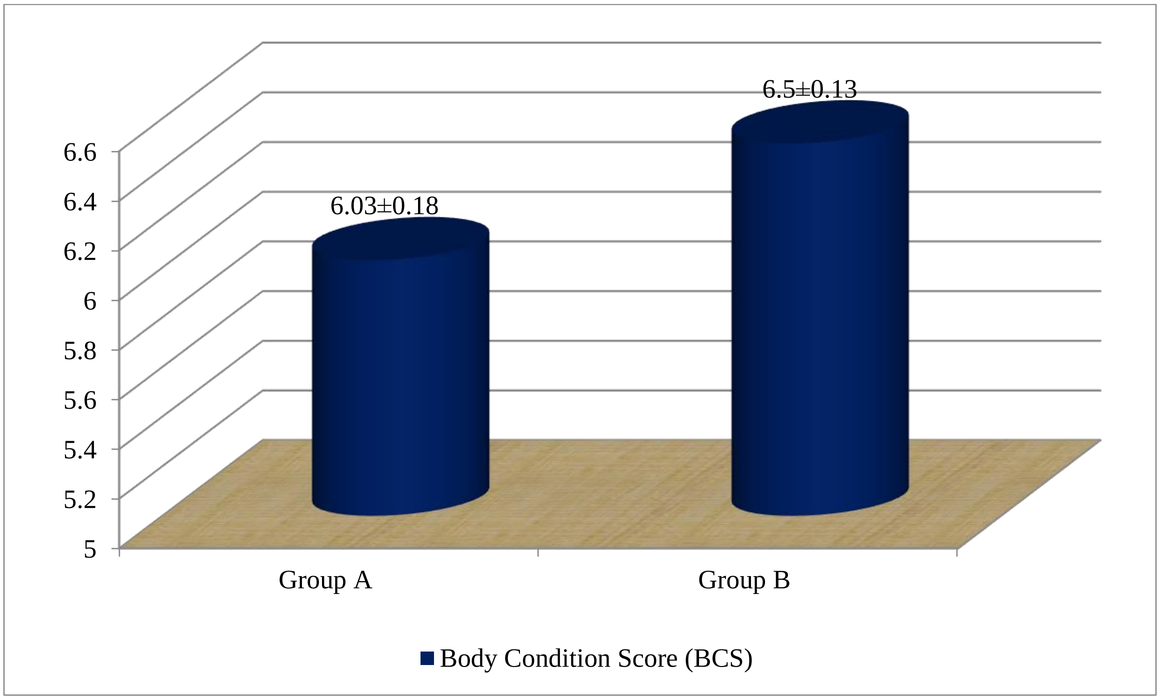
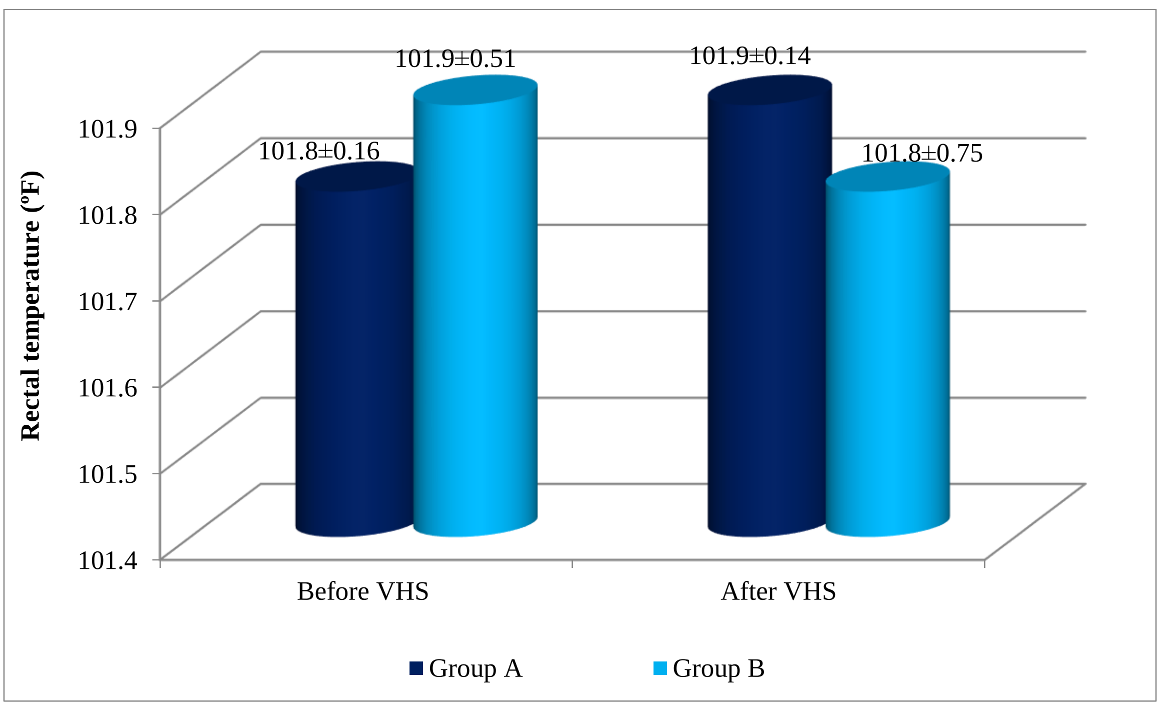


Fig. 6: Column showing the Mean ± SE values of rectal temperature in both Group A and B dogs before and after VHS evaluation



4.3.3.2 Heart rate (beats per minute)

The Mean ± SE values of heart rate in Group A dogs before VHS evaluation was 101 ± 2.52 beats per minute, which ranged between 94 to 110 beats per minute and the same after VHS evaluation was 102.6 ± 2.01 beats per minute, which ranged between 97 to 110 beats per minute. The Mean ± SE values of heart rate in Group B dogs before VHS evaluation was 120.3 ± 4.72 beats per minute, which ranged between 109 to 140 beats per minute and the same after VHS evaluation was 122.3 ± 4.49 beats per minute, which ranged between 110 to 136 beats per minute. The variations in the heart rate were within the normal physiological range in Group A dogs. These variations were statistically non-significant within both the groups (P value ≥ 0.05), but the variation in the heart rate between Group A and Group B dogs was statistically significant (P value ≤ 0.05) **(Fig. 7).**

4.3.3.3 Respiratory rate (breaths per minute)

The Mean ± SE values of respiratory rate in Group A dogs before VHS evaluation was 28.83 ± 0.94 breaths per minute, which ranged between 26 to 32 breaths per minute and the same after VHS evaluation was 30.1 ± 1.01 breaths per minute, which ranged between 27 to 34 breaths per minute. The Mean ± SE values of respiratory rate in Group B dogs before VHS evaluation was 46.5 ± 2.71 breaths per minute, which ranged between 37 to 56 breaths per minute and the same after VHS evaluation was 45.66 ± 2.44 breaths per minute, which ranged between 35 to 52 breaths per minute. The variations in the respiratory rate were within the normal physiological range in Group A dogs.

Fig. 7: Column showing the Mean ± SE values of heart rate in both Group A and B dogs before and after VHS evaluation

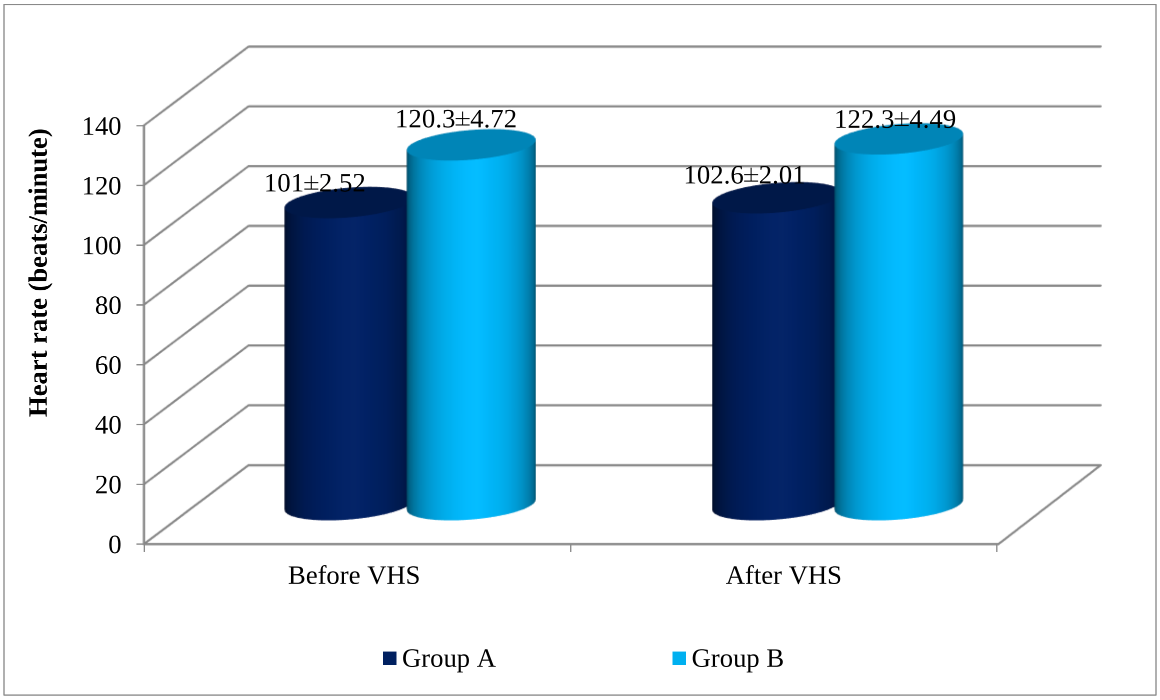
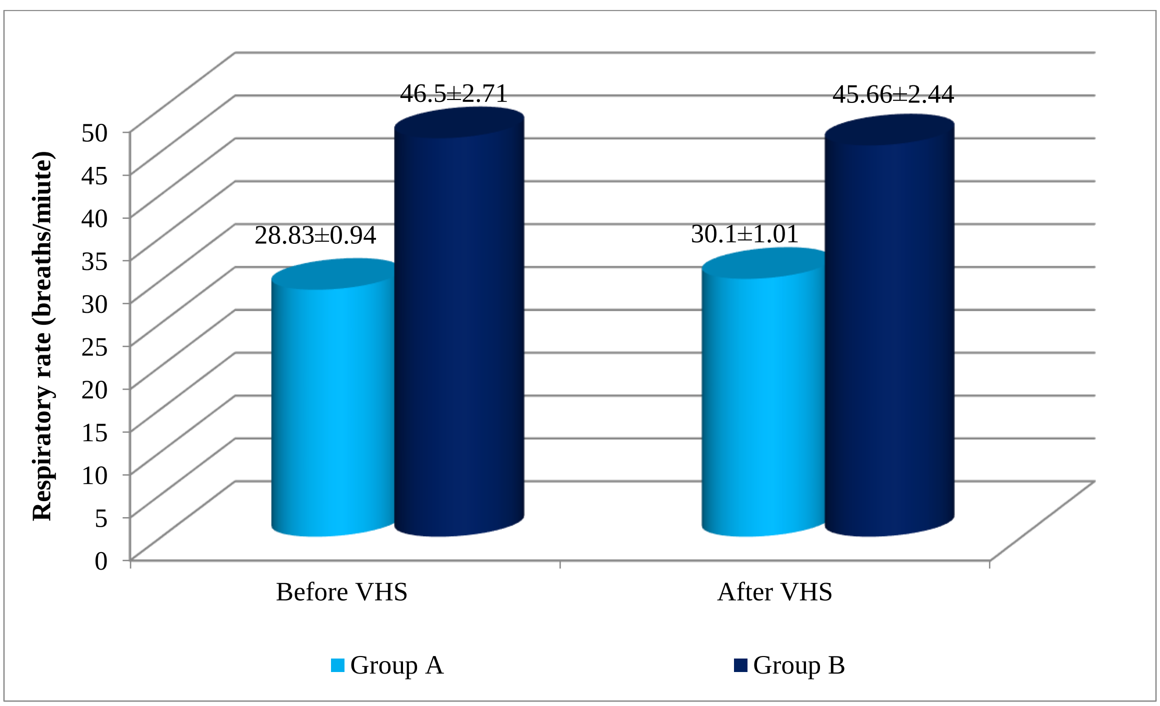


Fig. 8: Column showing the Mean ± SE values of respiratory rate in both Group A and B dogs before and after VHS evaluation



These variations were statistically non-significant within both the groups (P value ≥ 0.05), but the variation in the respiratory rate between Group A and Group B dogs was statistically significant (P value ≤ 0.05) **(Fig. 8).**

4.3.3.4 Blood pressure (mmHg)

The Mean ± SE values of systolic blood pressure (mmHg) and diastolic blood pressure (mmHg) in Group A dogs before VHS evaluation was 122.5 ± 1.85 mmHg and 79.66 ± 0.84 mmHg respectively. These values ranged between 115 to 128 mmHg and 77 to 82 mmHg respectively. Similarly the Mean ± SE values of systolic blood pressure (mmHg) and diastolic blood pressure (mmHg) in Group B dogs before VHS evaluation was 143.1 ± 5.57 mmHg and 82.33 ± 2.13 mmHg respectively and they ranged between 128 to 162 mmHg and 78 to 92 mmHg respectively. The Mean ± SE values of systolic blood pressure (mmHg) and diastolic blood pressure (mmHg) in Group A dogs after VHS evaluation was 123.66 ± 1.28 mmHg and 80.33 ± 0.84 mmHg respectively. These values ranged between 120 to 129 mmHg and 78 to 84 mmHg respectively. Similarly the Mean ± SE values of systolic blood pressure (mmHg) and diastolic blood pressure (mmHg) in Group B dogs after VHS evaluation was 142.66 ± 5.12 mmHg and 82 ± 1.94 mmHg respectively and they ranged between 125 to 158 mmHg and 75 to 90 mmHg respectively.

The variations in the Mean ± SE values of both systolic blood pressure (mmHg) and diastolic blood pressure (mmHg) in Group A dogs before and after VHS evaluation were within normal physiological range. But in case of Group B dogs the systolic blood pressure (mmHg) was slightly higher than normal physiological range and diastolic blood pressure (mmHg) was within normal physiological range **(Fig. 9 and Fig. 10).**

Fig. 9: Column showing the Mean ± SE values of systolic blood pressure (mmHg) in both Group A and B dogs before and after VHS evaluation

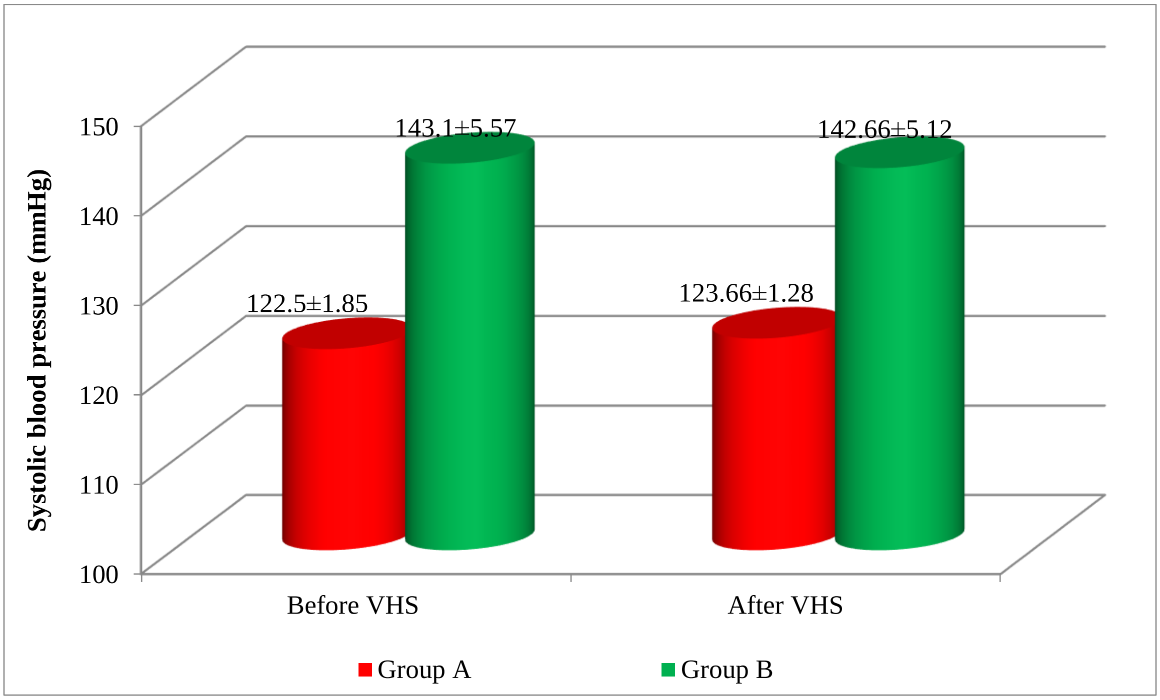


Fig. 10: Column showing the Mean ± SE values of diastolic blood pressure (mmHg) in both Group A and B dogs before and after VHS evaluation

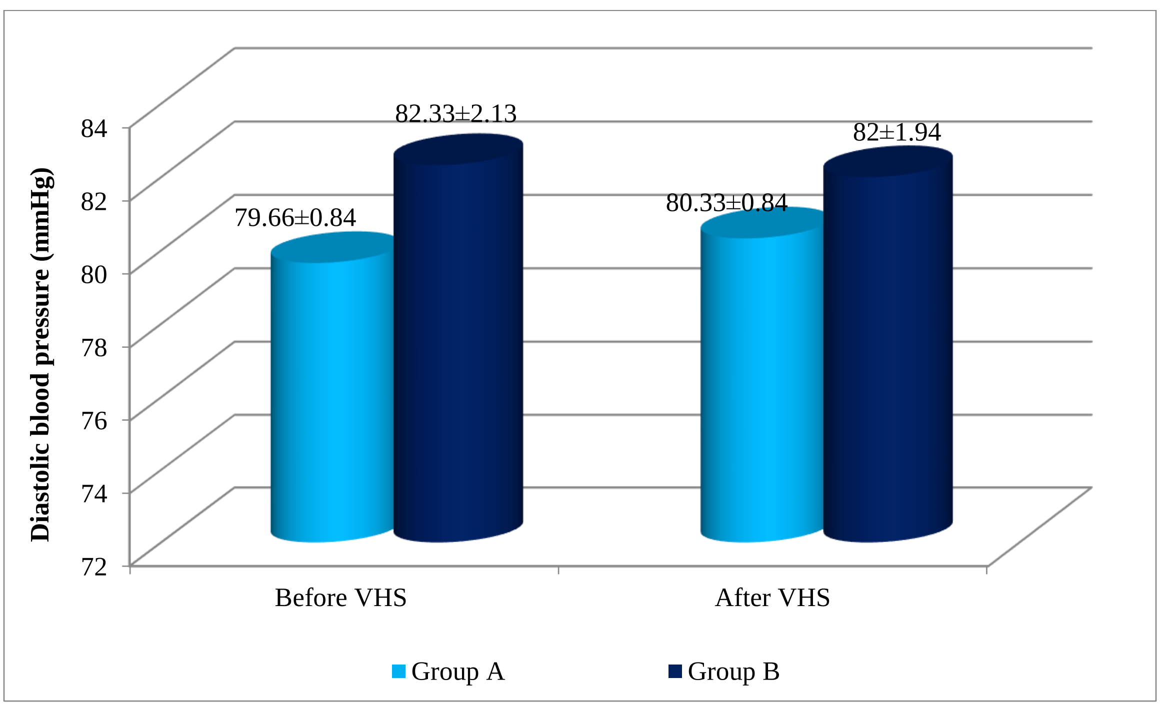


Table 8: The Mean ± SE values of physiological parameters in both Group A and B dogs before and after VHS evaluation

Physiological parameters	Group A		Group B		P value between the groups
	Before	After	Before	After	
Rectal temperature (ºF)	101.8±0.16	101.9±0.14	101.9±0.51	101.8±0.75	0.51[b] 0.96[a]
P value within the groups	0.63		0.32		
Heart rate (beats per minute)	101±2.52	102.6±2.01	120.3±4.72	122.3±4.49	0.004[b*] 0.002[a*]
P value within the groups	0.16		0.47		
Respiratory rate (breaths per minute)	28.83±0.94	30.1±1.01	46.5±2.71	45.66±2.44	0.001[b*] 0.001[a*]
P value within the groups	0.4		0.38		
Systolic blood pressure (mmHg)	122.5±1.85	123.66±1.28	143.1±5.57	142.66±5.12	0.005[b*] 0.004[a*]
P value within the groups	0.28		0.71		
Diastolic blood pressure (mmHg)	79.66±0.84	80.33±0.84	82.33±2.13	82±1.94	0.27[b] 0.45[a]
P value within the groups	0.53		0.74		

* represents statistical significance between the groups (P value < 0.05)

[b] represents P value between the groups before VHS evaluation

[a] represents P value between the groups after VHS evaluation

These variations were statistically non-significant within both the groups (P value ≥ 0.05), but the variation in the systolic blood pressure (mmHg) between Group A and Group B dogs was statistically significant (P value ≤ 0.05) that of diastolic blood pressure (mmHg) was statistically non-significant between the groups (P value ≥ 0.05).

4.3.4 Haematological parameters

The Mean ± SE values of the following haematological parameters of both groups before and after VHS evaluation were shown in **Table 9.**

4.3.4.1 Total erythrocyte count (TEC) (millions/cmm)

The Mean ± SE values of total erythrocyte count (TEC) (millions/cmm) in Group A dogs before and after VHS evaluation was 6.85 ± 0.49 millions/cmm and 7.005 ± 0.43 millions/cmm, these values ranged between 5.37 to 8.79 millions/cmm and 5.6 to 8.7 millions/cmm respectively.

The Mean ± SE values of total erythrocyte count (TEC) (millions/cmm) in Group B dogs before and after VHS evaluation was 6.81 ± 0.093 millions/cmm and 6.94 ± 0.06 millions/cmm, these values ranged between 6.48 to 7.1 millions/cmm and 6.71 to 7.12 millions/cmm respectively.

The variations in Mean ± SE values of total erythrocyte count (TEC) (millions/cmm) were statistically non-significant both within and between the groups (P value ≥ 0.05) **(Fig. 11).**

4.3.4.2 Total leukocyte count (TLC) (thousands/cmm)

The Mean ± SE values of total leukocyte count (TLC) (thousands/cmm) in Group A dogs before and after VHS evaluation was 12 ± 0.84 thousands/cmm and 12.41 ± 0.87 thousands/cmm, these values ranged between 9.6 to 15.1and 9.2 to 15.5 thousands/cmm respectively. The same in Group B dogs before and after VHS evaluation was 12.51 ± 0.8 thousands/cmm and 12.66 ± 0.68 thousands/cmm, these values ranged between 10.8 to 16.3 and 11.3 to 15.9 thousands/cmm respectively.

The Mean ± SE values of TLC varied non significantly both within and between the groups (P value ≥ 0.05) **(Fig. 12).**

4.3.4.3 Hemoglobin (g/dL)

The Mean ± SE values of haemoglobin (g/dL) in Group A dogs before and after VHS evaluation was 13.38 ± 0.26 g/dL and 13.15 ± 0.32 g/dL, these values ranged between 12.1 to 13.8 g/dL and 11.6 to 13.8 g/dL respectively. The same in Group B dogs before and after VHS evaluation was 12.88 ± 0.09 g/dL and 12.91 ± 0.14 g/dL, these values ranged between 12.6 to 13.2 g/dL and 12.4 to 13.4 g/dL respectively.

The Mean ± SE values of haemoglobin (g/dL) varied non significantly both within and between the groups (P value ≥ 0.05) **(Fig. 13).**

4.3.4.4 Packed cell volume (PCV) (%)

The Mean ± SE values of PCV (%) in Group A dogs before and after VHS evaluation was 39.3 ± 0.19 % and 39.5 ± 0.29 %, these values ranged between 38.5 to 39.9 % and 38.6 to 40.5 % respectively. The same in Group B dogs before and after VHS

Fig. 11: Column showing the Mean ± SE values of total erythrocyte count (TEC) (millions/cmm) in both Group A and B dogs before and after VHS evaluation

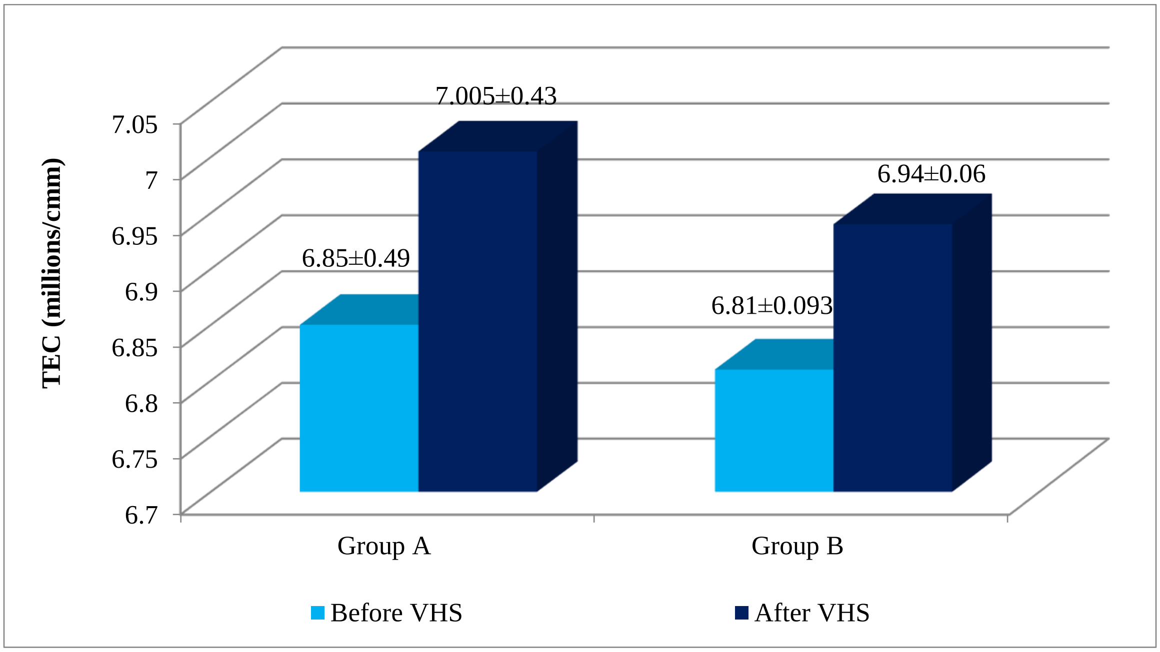


Fig. 12: Column showing the Mean ± SE values of total leukocyte count (TLC) (thousands/cmm) in both Group A and B dogs before and after VHS evaluation

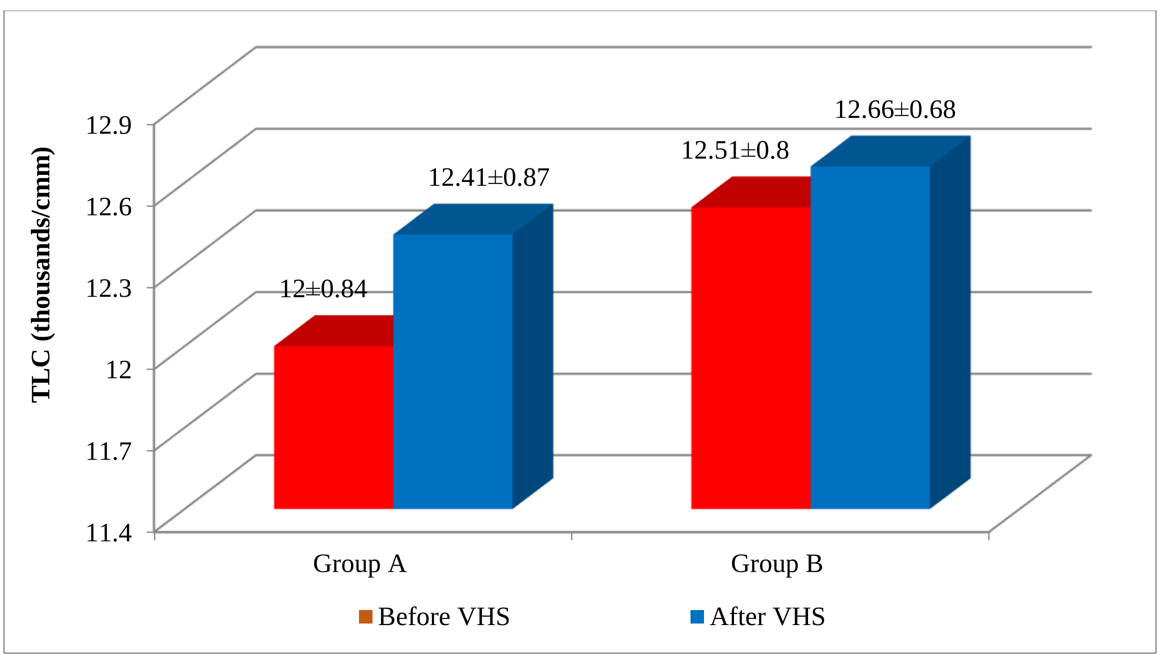


Fig. 13: Column showing the Mean ± SE values of haemoglobin (g/dL) in both Group A and B dogs before and after VHS evaluation

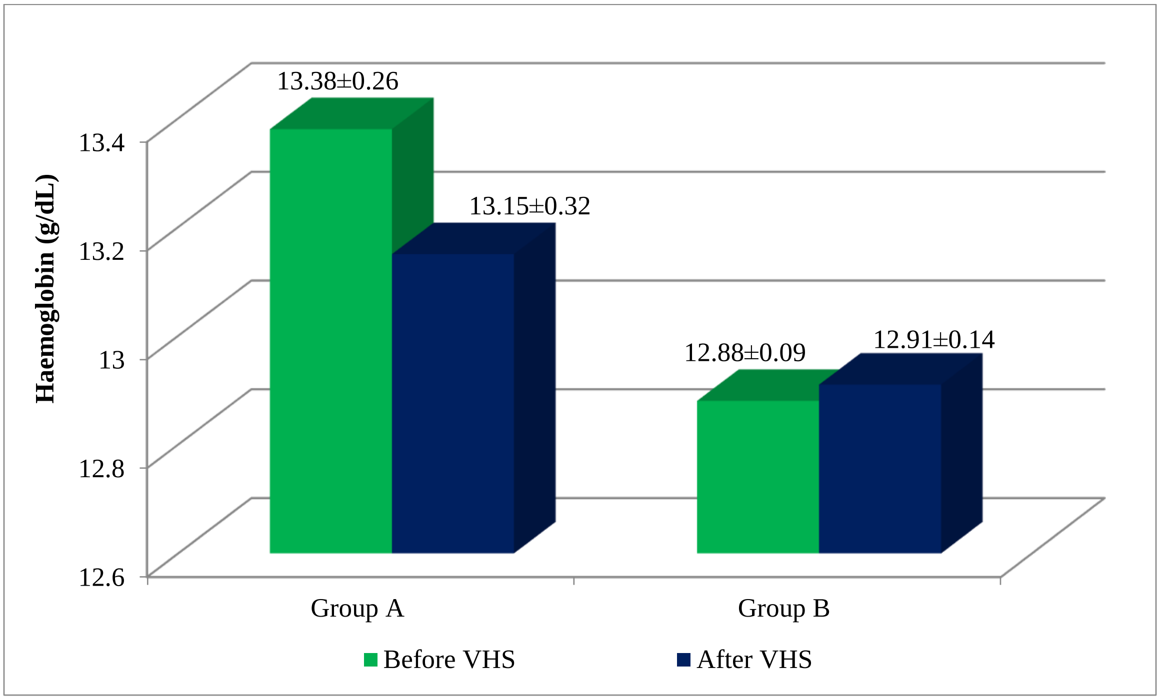
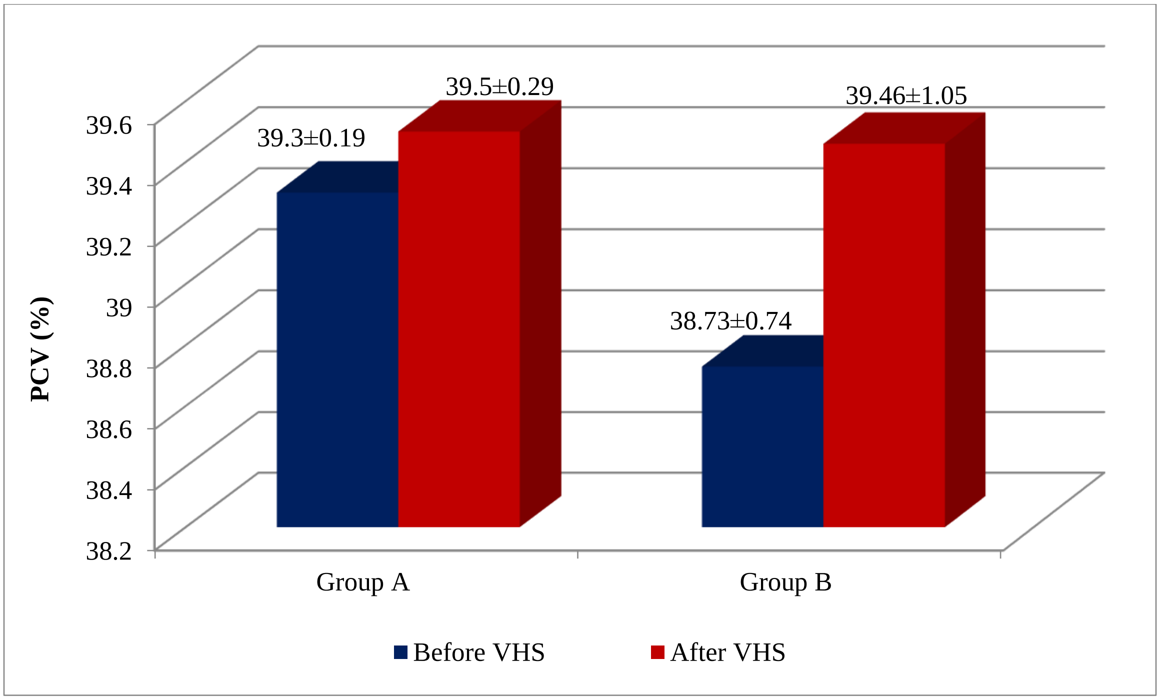


Fig. 14: Column showing the Mean ± SE values of PCV (%) in both Group A and B dogs before and after VHS evaluation



evaluation was 38.73 ± 0.74 % and 39.46 ± 1.05 %, these values ranged between 36.6 to 41 % and 36.8 to 44 % respectively.

The Mean ± SE values PCV (%) varied non significantly both within and between the group A and B dogs (P value ≥ 0.05) **(Fig. 14).**

4.3.4.5 Differential Leukocyte Count (DLC)

The Mean ± SE values of the following DLC of both groups before and after VHS evaluation were shown in **Fig. 15.**

4.3.4.5.1 Neutrophils (%)

The Mean ± SE values of neutrophils in Group A dogs before VHS evaluation was 71.7 ± 1.07 %, which ranged between 67.8 to 75 % and the same after VHS evaluation was 71.8 ± 1.08 %, which ranged between 68 to 76 %.

The Mean ± SE values of neutrophils in Group B dogs before VHS evaluation was 75.05 ± 2.53 %, which ranged between 69.8 to 85 % and the same after VHS evaluation was 75.1 ± 2.79 %, which ranged between 68 to 87 %.

The variations in the Mean ± SE values of neutrophils were statistically non-significant both within and between the groups (P value ≥ 0.05).

4.3.4.5.2 Lymphocytes (%)

The Mean ± SE values of lymphocytes in Group A dogs before VHS evaluation was 23.63 ± 0.73 %, which ranged between 21.5 to 25.2 % and the same after VHS evaluation was 23.75 ± 0.8 %, which ranged between 21 to 26 %.

The Mean ± SE values of lymphocytes in Group B dogs before VHS evaluation was 21.8 ± 2.18 %, which ranged between 14 to 27 % and the same after VHS evaluation was 21.23 ± 2.2 %, which ranged between 12 to 26 %.

The variations in the Mean ± SE values of lymphocytes were statistically non-significant both within and between the groups (P value ≥ 0.05).

4.3.4.5.3 Monocytes (%)

The Mean ± SE values of monocytes in Group A dogs before VHS evaluation was 2.46±0.13 %, which ranged between 2 to 3 % and the same after VHS evaluation was 2.48±0.18 %, which ranged between 2 to 3 %.

The Mean ± SE values of monocytes in Group B dogs before VHS evaluation was 2.1 ± 0.52 %, which ranged between 1 to 4.5 % and the same after VHS evaluation was 2.08 ± 0.37 %, which ranged between 1 to 3 %.

The variations in the Mean ± SE values of monocytes were statistically non-significant both within and between the groups (P value ≥ 0.05).

4.3.4.5.4 Eosinophils (%)

The Mean ± SE values of eosinophils in Group A dogs before VHS evaluation was 1.95 ± 0.34 %, which ranged between 1 to 3 % and the same after VHS evaluation was 1.66 ± 0.21 %, which ranged between 1 to 2 %.

The Mean ± SE values of eosinophils in Group B dogs before VHS evaluation was 1.6 ± 0.89 %, which ranged between 0 to 6 % and the same after VHS evaluation was 1.11 ± 0.2 %, which ranged between 0 to 4 %.

The variations in the Mean ± SE values of eosinophils were statistically non-significant both within and between the groups (P value ≥ 0.05).

4.3.4.5.5 Basophils (%)

The Mean ± SE values of basophils in Group A dogs before VHS evaluation was 0.58 ± 0.27 %, which ranged between 0 to 1.5 % and the same after VHS evaluation was 0.43 ± 0.28 %, which ranged between 0 to 1.6 %.

The Mean ± SE values of basophils in Group B dogs before VHS evaluation was 0.31 ± 0.2 %, which ranged between 0 to 1 % and the same after VHS evaluation was 0.31 ± 0.2 %, which ranged between 0 to 1 %.

The variations in the Mean ± SE values of basophils were statistically non-significant both within and between the groups (P value ≥ 0.05).

4.3.5 Biochemical parameters

The Mean ± SE values of the following biochemical parameters of both groups before and after VHS evaluation were shown in **Table 10 and Fig. 16.**

Fig. 15: Column showing the Mean ± SE values of DLC (%) in both Group A and B dogs before and after VHS evaluation

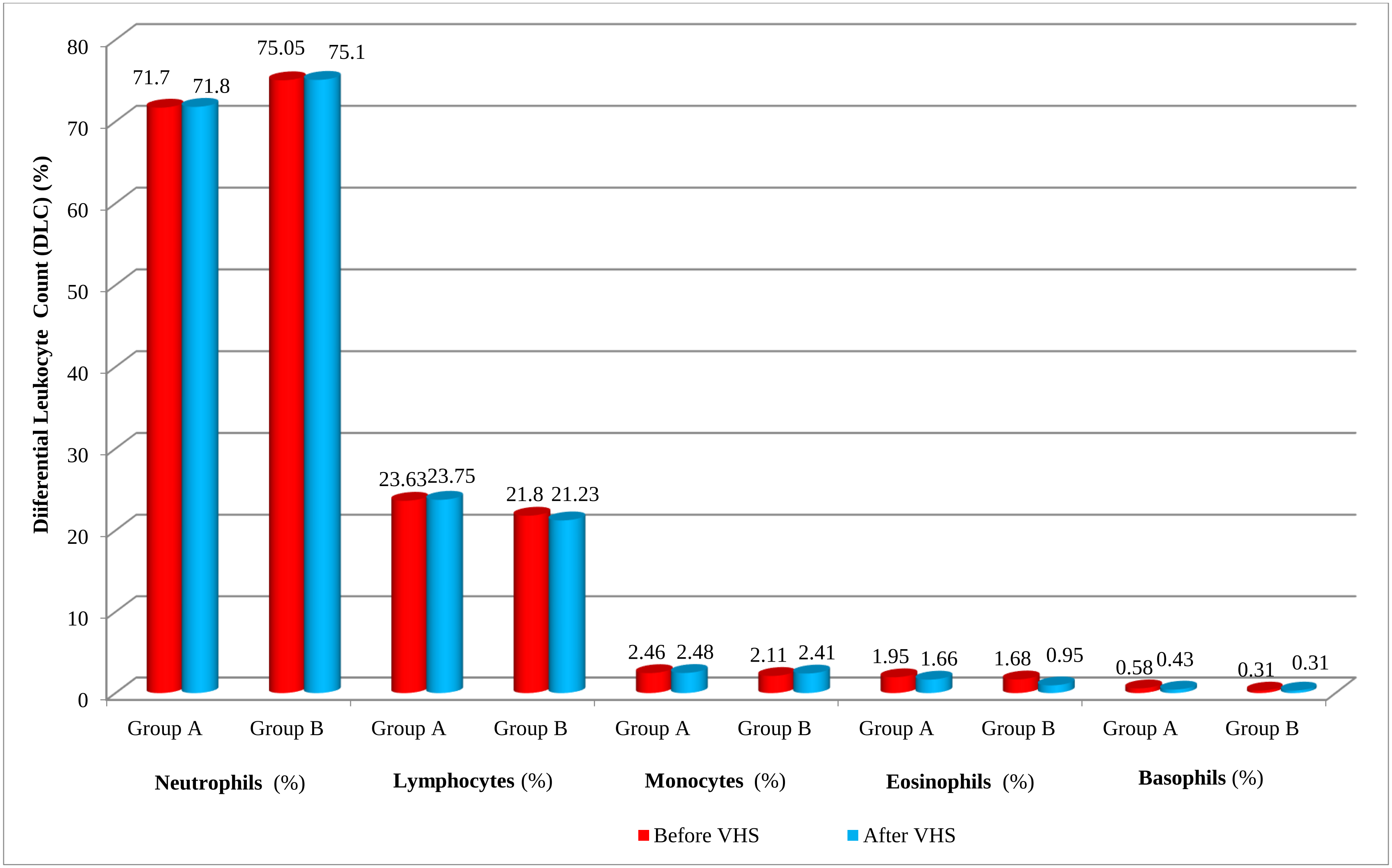


Table 9: The Mean ± SE values of haematological parameters in both Group A and B dogs before and after VHS evaluation

Physiological parameters	Group A		Group B		P value between the groups
	Before	After	Before	After	
Total Erythrocyte Count (TEC) (millions/cmm)	6.85±0.49	7.005±0.43	6.81±0.093	6.94±0.06	0.93[b]
P value within the groups	0.12		0.11		0.89[a]
Total Leukocyte Count (TLC) (thousands/cmm)	12±0.84	12.41±0.87	12.51±0.8	12.66±0.68	0.63[b]
P value within the groups	0.13		0.29		0.82[a]
Haemoglobin (g/dL)	13.38±0.26	13.15±0.32	12.88±0.09	12.91±0.14	0.1[b]
P value within the groups	0.07		0.73		0.52[a]
Packed Cell Volume (PCV) (%)	39.3±0.19	39.5±0.29	38.73±0.74	39.46±1.05	0.46[b]
P value within the groups	0.49		0.28		0.96[a]
Neutrophils (%)	71.7±1.07	71.8±1.08	75.05±2.53	75.1±2.79	0.25[b]
P value within the groups	0.78		0.94		0.30[a]
Lymphocytes (%)	23.63±0.73	23.75±0.8	21.8±2.18	21.23±2.2	0.45[b]
P value within the groups	0.82		0.35		0.30[a]
Monocytes (%)	2.46±0.13	2.48±0.18	2.1±0.52	2.08±0.37	0.53[b]
P value within the groups	0.91		0.92		0.35[a]
Eosiniphils (%)	1.95±0.34	1.66±0.21	1.6±0.89	1.11±0.2	0.78[b]
P value within the groups	0.21		0.5		0.14[a]
Basophils (%)	0.58±0.27	0.43±0.28	0.31±0.2	0.31±0.2	0.44[b]
P value within the groups	0.41		0.5		0.74[a]

[b] represents P value between the groups before VHS evaluation

[a] represents P value between the groups after VHS evaluation

4.3.5.1 Serum creatinine (mg/dL)

The Mean ± SE values of serum creatinine (mg/dL) in Group A dogs before VHS evaluation was 1.02 ± 0.16 mg/dL, which ranged between 0.5 to 1.5 mg/dL and the same after VHS evaluation was 1.03 ± 0.16 mg/dL, which ranged between 0.49 to 1.5 mg/dL.

The Mean ± SE values of serum creatinine (mg/dL) in Group B dogs before VHS evaluation was 1.47 ± 0.34 mg/dL, which ranged between 0.62 to 2.95 mg/dL and the same after VHS evaluation was 1.43 ± 0.31 mg/dL, which ranged between 0.72 to 2.85 mg/dL.

The variations in the Mean ± SE values of serum creatinine (mg/dL) were statistically non-significant both within and between the groups (P value ≥ 0.05).

4.3.5.2 Blood urea nitrogen (BUN) (mg/dL)

The Mean ± SE values of Blood urea nitrogen (BUN) (mg/dL) in Group A dogs before VHS evaluation was 12.2 ± 1.3 mg/dL, which ranged between 7.42 to 15.5 mg/dL and the same after VHS evaluation was 10.84 ± 2.1mg/dL, which ranged between 8.2 to 15.5 mg/dL.

The Mean ± SE values of Blood urea nitrogen (BUN) (mg/dL) in Group B dogs before VHS evaluation was 13.11 ± 0.62 mg/dL, which ranged between 10.1 to 14.2 mg/dL and the same after VHS evaluation was 13.3 ± 0.77 mg/dL, which ranged between 9.5 to 14.6 mg/dL. The variations in the Mean ± SE values of Blood urea nitrogen (BUN) (mg/dL) were statistically non-significant both within and between the groups (P value ≥ 0.05).

4.3.5.3 Alanine amino transferase (ALT) (IU/L)

The Mean ± SE values of Alanine amino transferase (ALT) in Group A dogs before VHS evaluation was 30.23 ± 5.9 IU/L, which ranged between 12.5 to 49 IU/L and the same after VHS evaluation was 29.7 ± 6.4 IU/L, which ranged between 11.5 to 50 IU/L.

The Mean ± SE values of Alanine amino transferase (ALT) in Group B dogs before VHS evaluation was 31.74 ± 2.9 IU/L, which ranged between 22 to 42 IU/L and the same after VHS evaluation was 31.2 ± 3.6 IU/L, which ranged between 19.7 to 44 IU/L.

The variations in the Mean ± SE values of Alanine amino transferase (ALT) (IU/L) were statistically non-significant both within and between the groups (P value ≥ 0.05).

4.3.6 Diagnostic parameters

4.3.6.1 Thoracic radiography

In Group A, the cardiac silhouette appeared to be oval or egg shape in 83.33% of dogs (5 out of 6) in right lateral recumbency whereas in left lateral recumbency, it became more circular in shape in 66.67% of dogs (4 out of 6) and occupied 3 intercostal space in 66.67% (4 out of 6) and the cardiac silhouette of remaining two dogs occupied 3.5 intercostal space on both lateral thoracic radiographs **(Plate 9 to 10 & 13 to 22).**

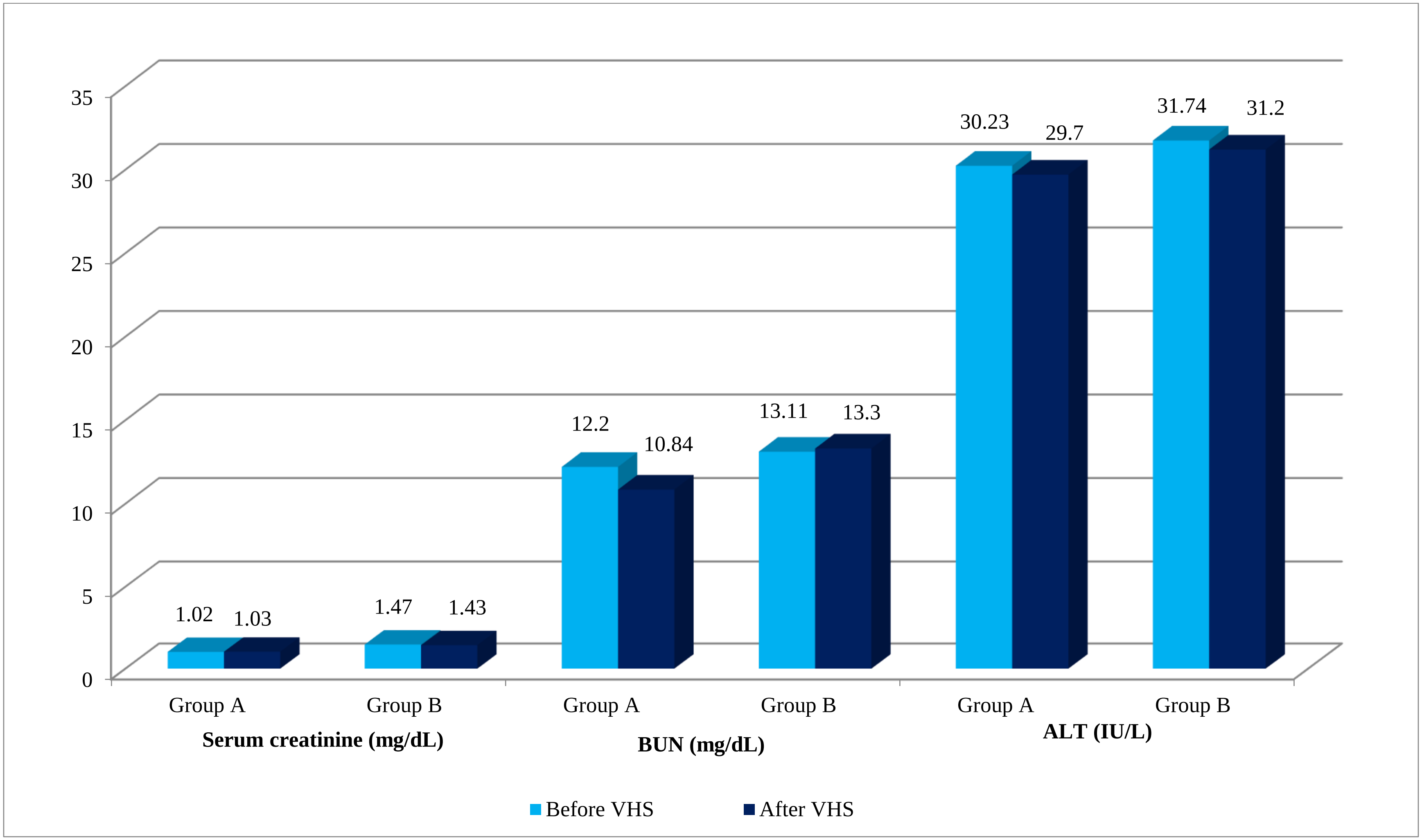
Table 10: The Mean ± SE values of biochemical parameters in both Group A and B dogs before and after VHS evaluation

Physiological parameters	Group A		Group B		P value between the groups
	Before	After	Before	After	
Serum creatinine (mg/dL)	1.02±0.16	1.03±0.16	1.47±0.34	1.43±0.31	0.26[b]
P value within the groups	0.88		0.44		0.28[a]
Blood urea nitrogen (BUN) (mg/dL)	12.2±1.3	10.84±2.1	13.11±0.62	13.3±0.77	0.55[b]
P value within the groups	0.4		0.33		0.30[a]
Alanine amino transferase (ALT) (IU/L)	30.23±5.9	29.7±6.4	31.74±2.9	31.2±3.6	0.82[b]
P value within the groups	0.45		0.62		0.84[a]

[b] represents P value between the groups before VHS evaluation

[a] represents P value between the groups after VHS evaluation

Fig. 16: Column showing the Mean ± SE values of biochemical parameters in both Group A and B dogs before and after VHS evaluation



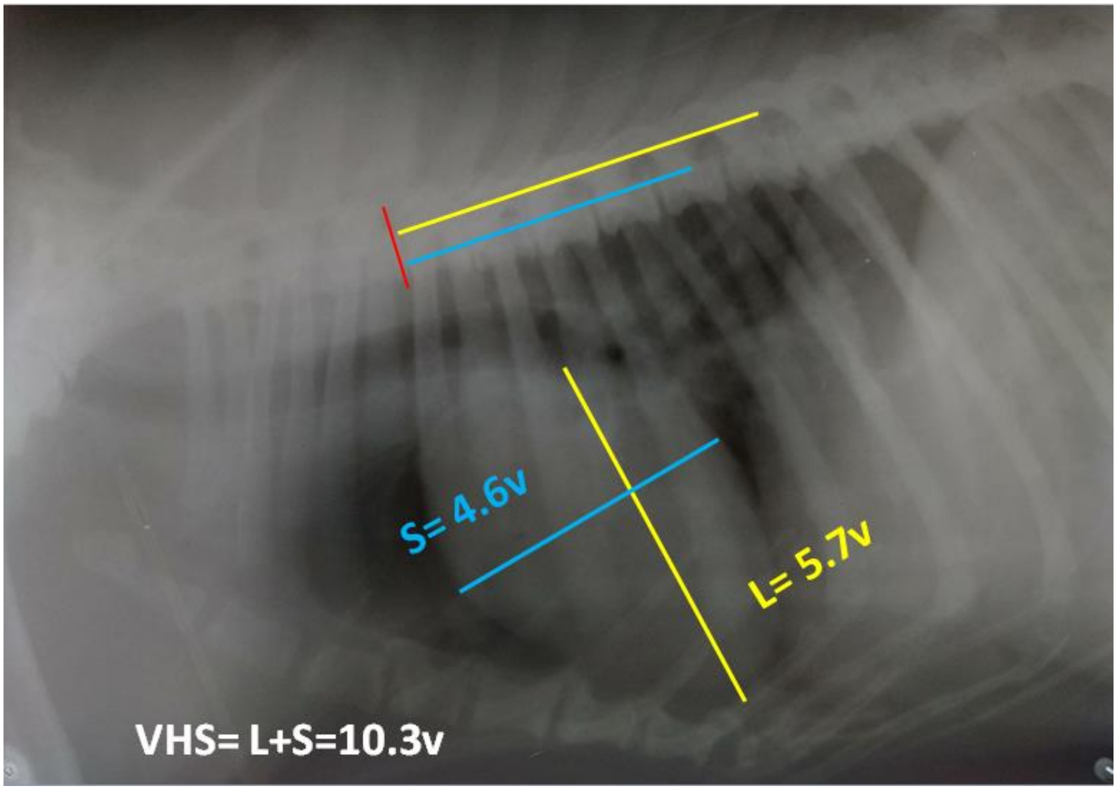
In Group B, the dog with biventricular enlargement showed the rounding of the cardiac silhouette with increased sternal contact **(Plate 23 and 24).** The dogs with right ventricular enlargement showed increased sternal contact, tilting and convexity of cranial margin of cardiac shadow in both right and left lateral thoracic projections. There was also the presence of cardiac bulging from 6 O' clock to 9 O' clock position **(Plate 25 to 30).** The dog with right atrial enlargement showed elevated trachea, cardiac bulging from 10 o' clock to 11 O' clock position and rounding of cardiac silhouette in left lateral thoracic radiograph **(Plate 31 and 32).** In left atrio-ventricular enlargement, there was loss of cardiac waist, cardiac bulging from 1 O' clock to 2 O' clock and 3 O' clock to 6 O' clock position, elevation of carina and trachea appeared parallel to thoracic spine **(Plate 33 and 34)**.

4.3.6.2 Vertebral Heart Score evaluation

The Vertebral Heart Score was calculated in dogs of both the groups on both right and left lateral thoracic radiographs. The VHS calculation in Group A dogs was shown from **Plate 9 to 10 & 13 to 22** and the same for Group B dogs was shown from **Plate 23 to 34.**

4.3.6.2.1 Vertebral Heart Score evaluation in Group A dogs

The Mean ± SE values of VHS in Group A dogs on right lateral thoracic radiograph was 10.1 ± 0.03v, which ranged between 10.1 to 10.3v and the same on left lateral thoracic radiograph was 9.8 ± 0.03v, which ranged between 9.7 to 9.9v.



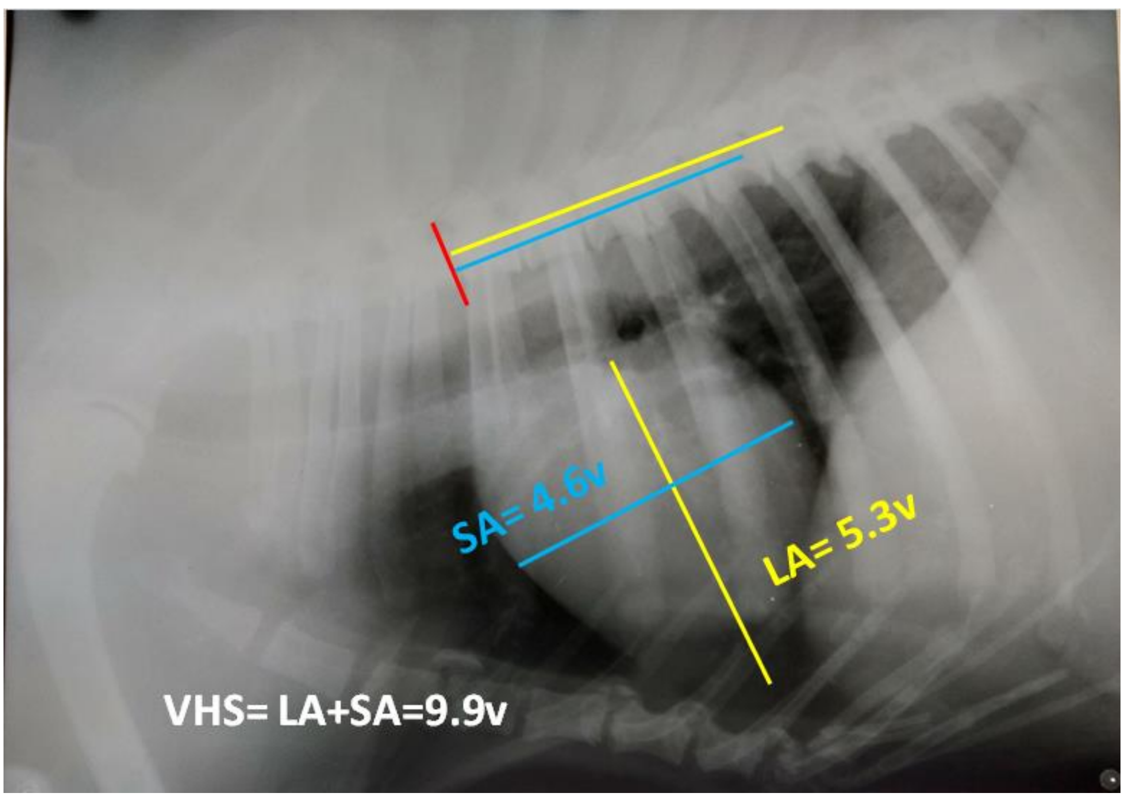
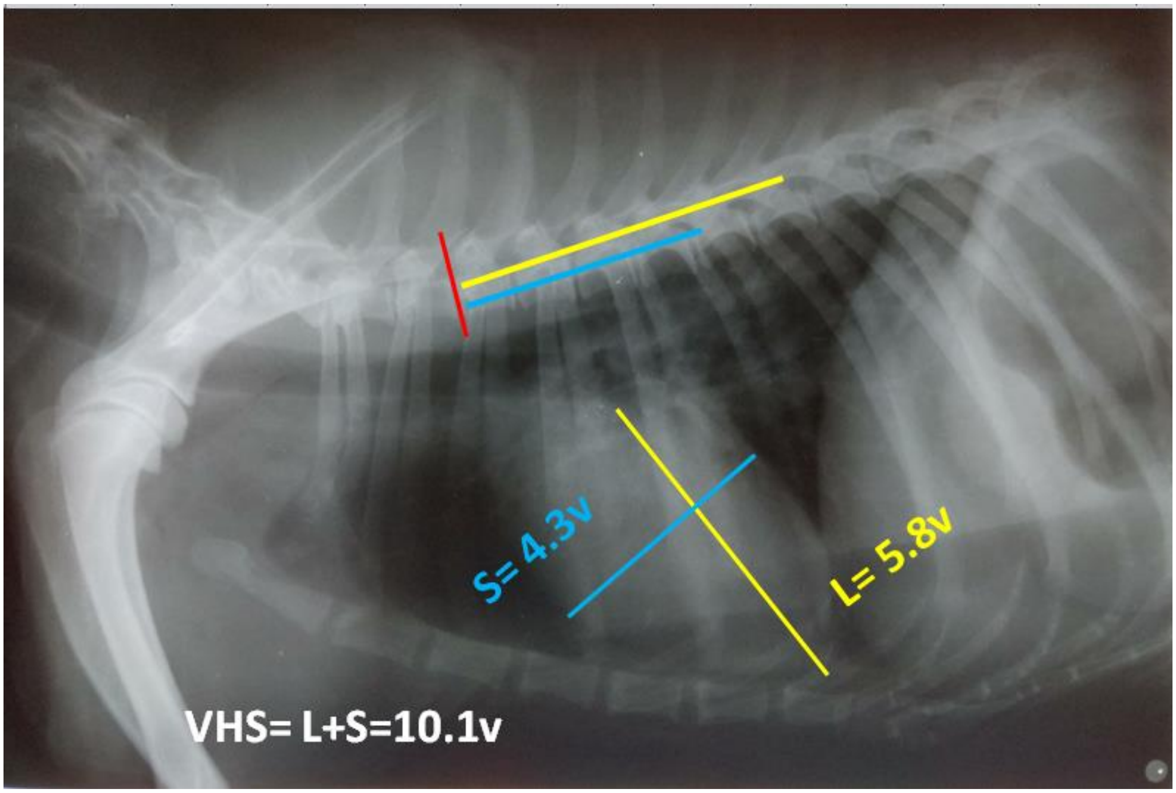


Plate 13 & 14: R-VHS and L-VHS of dog 2 (Group A) respectively.



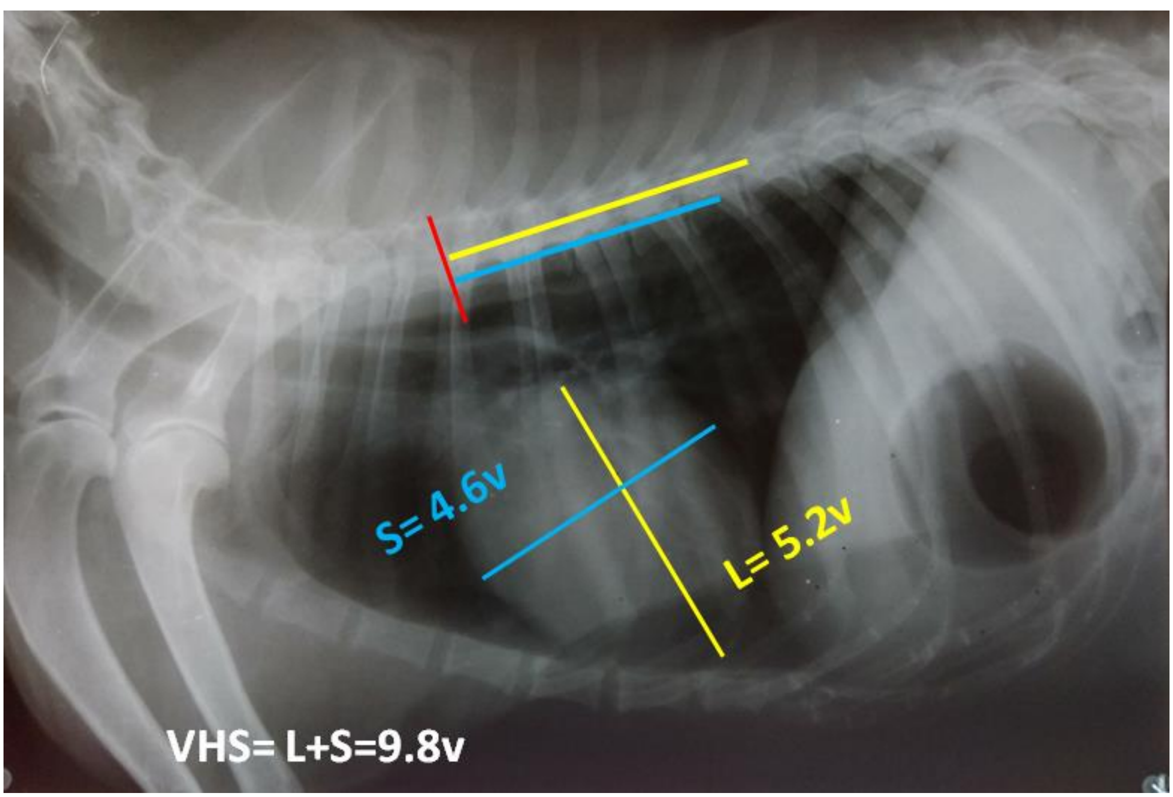
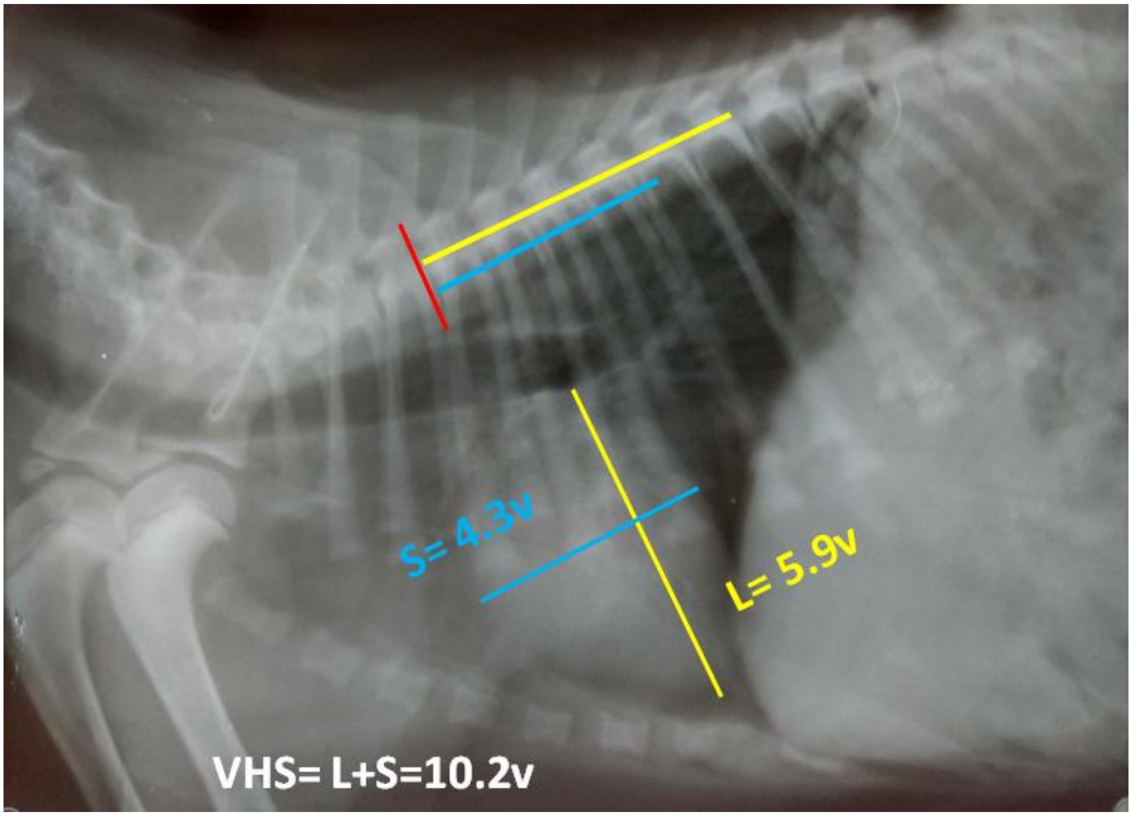


Plate 15 & 16: R-VHS and L-VHS of dog 3 (Group A) respectively.



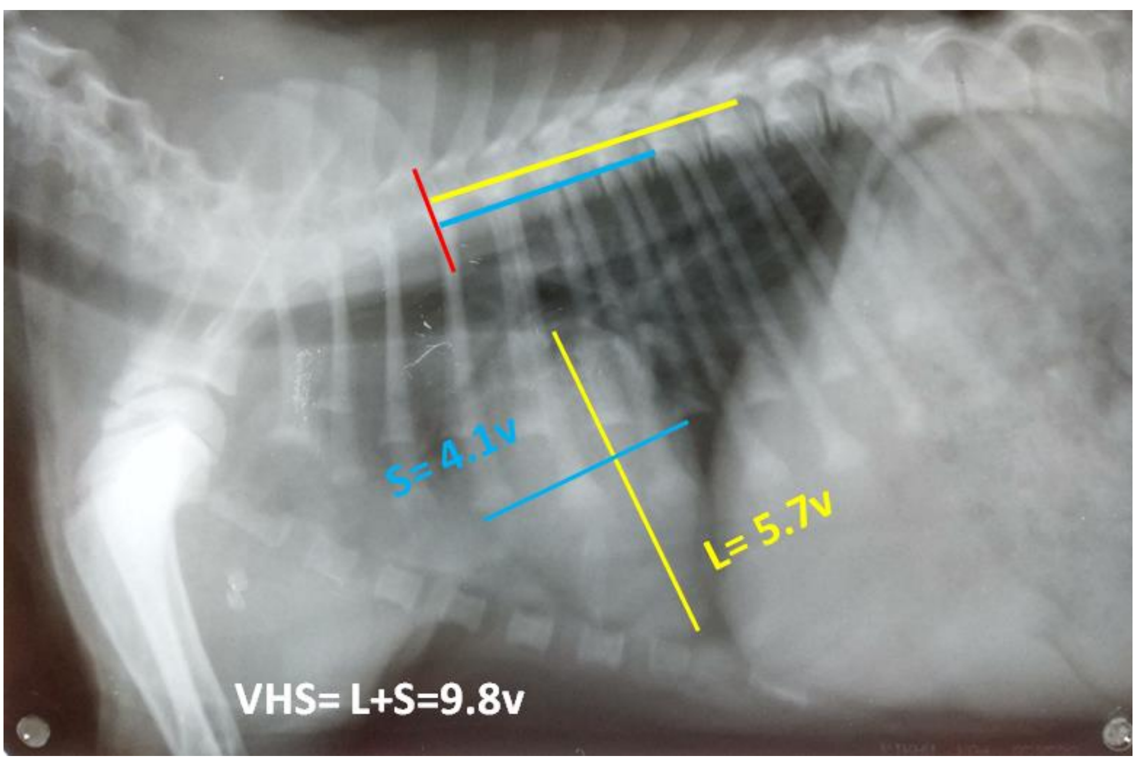
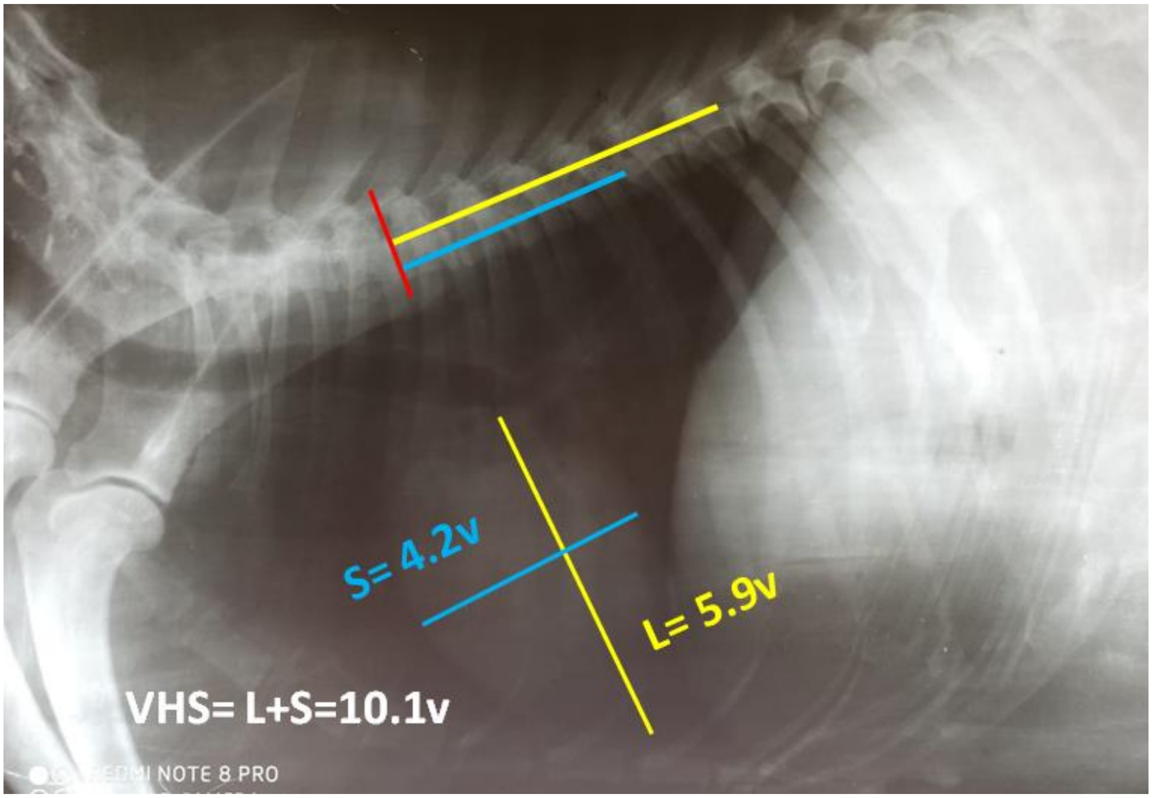


Plate 17 & 18: R-VHS and L-VHS of dog 4 (Group A) respectively.



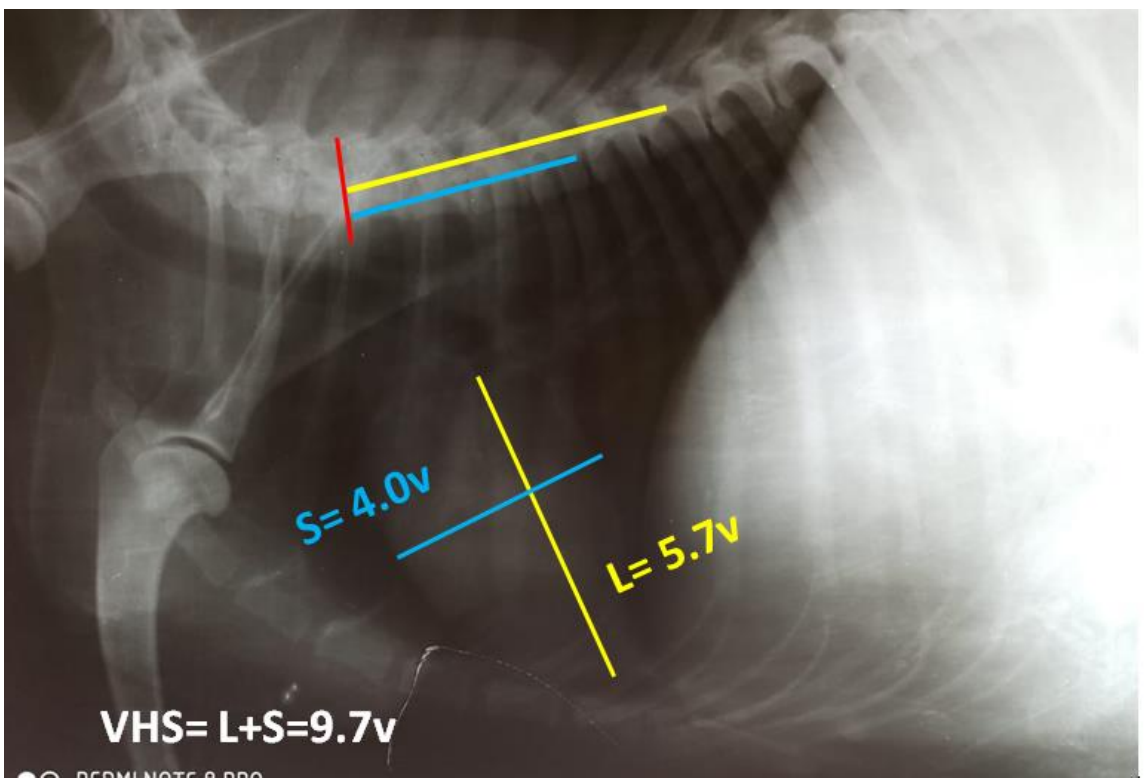
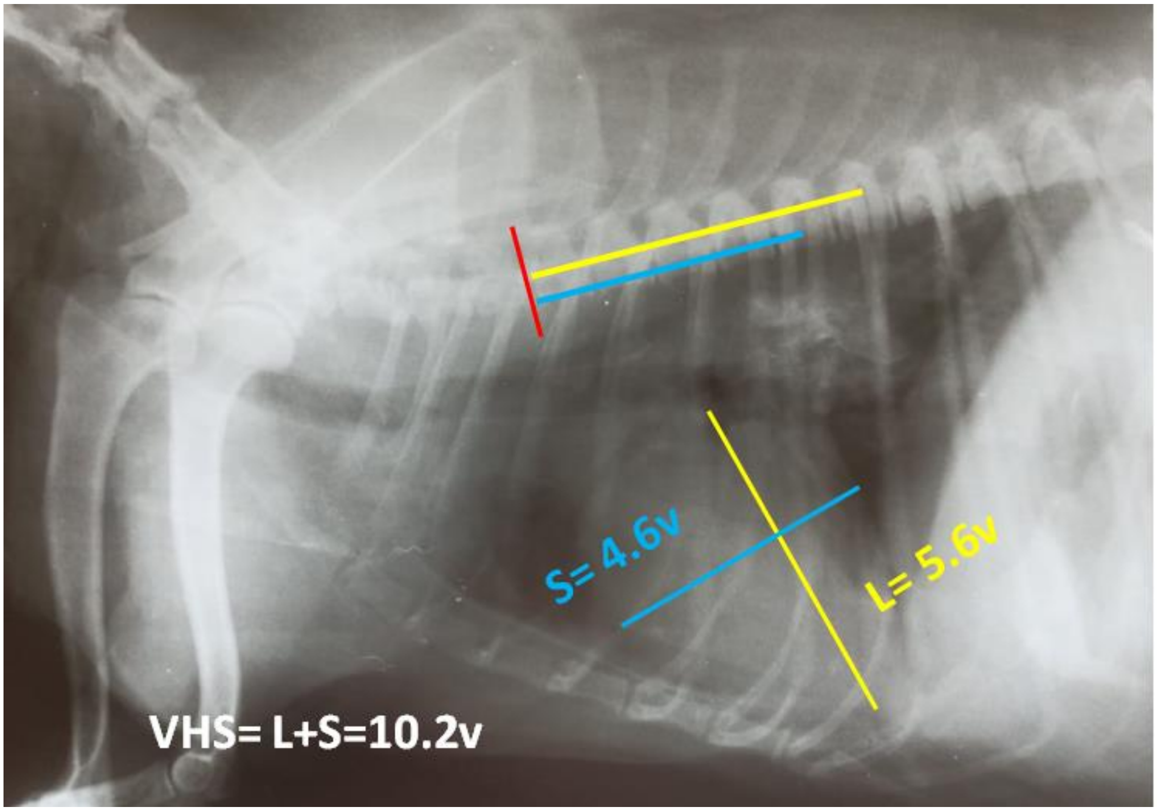


Plate 19 & 20: R-VHS and L-VHS of dog 5 (Group A) respectively.



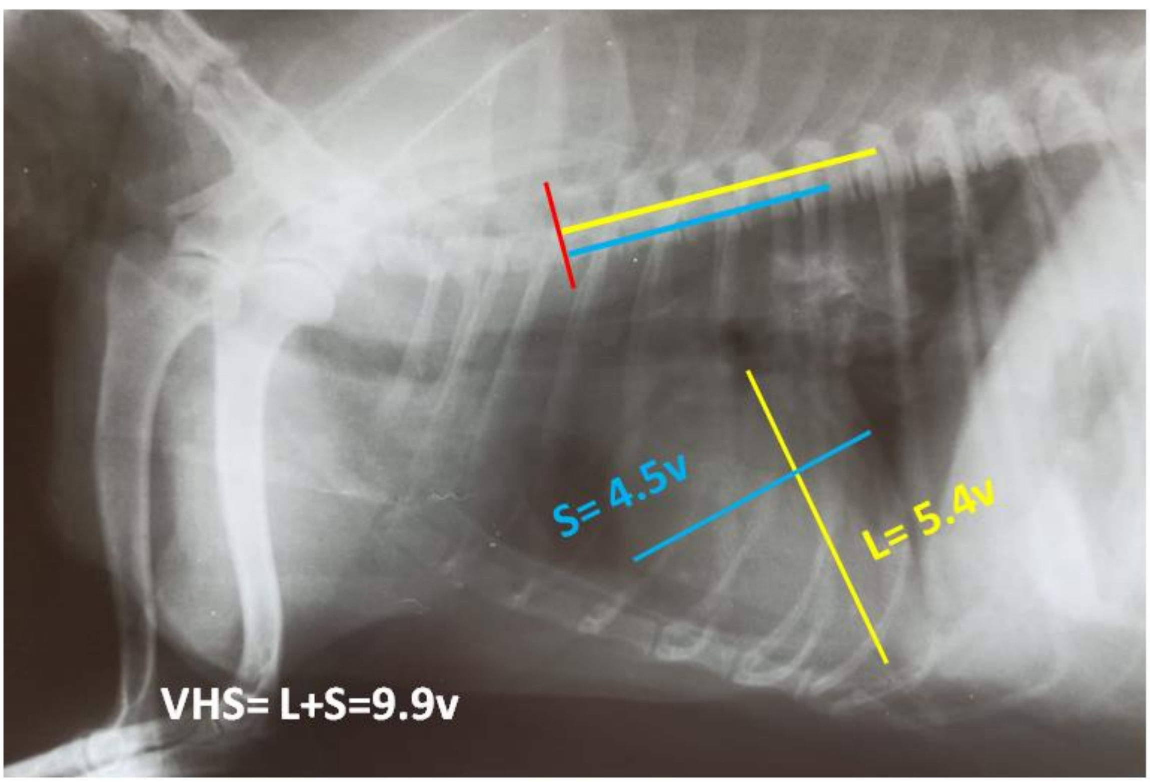
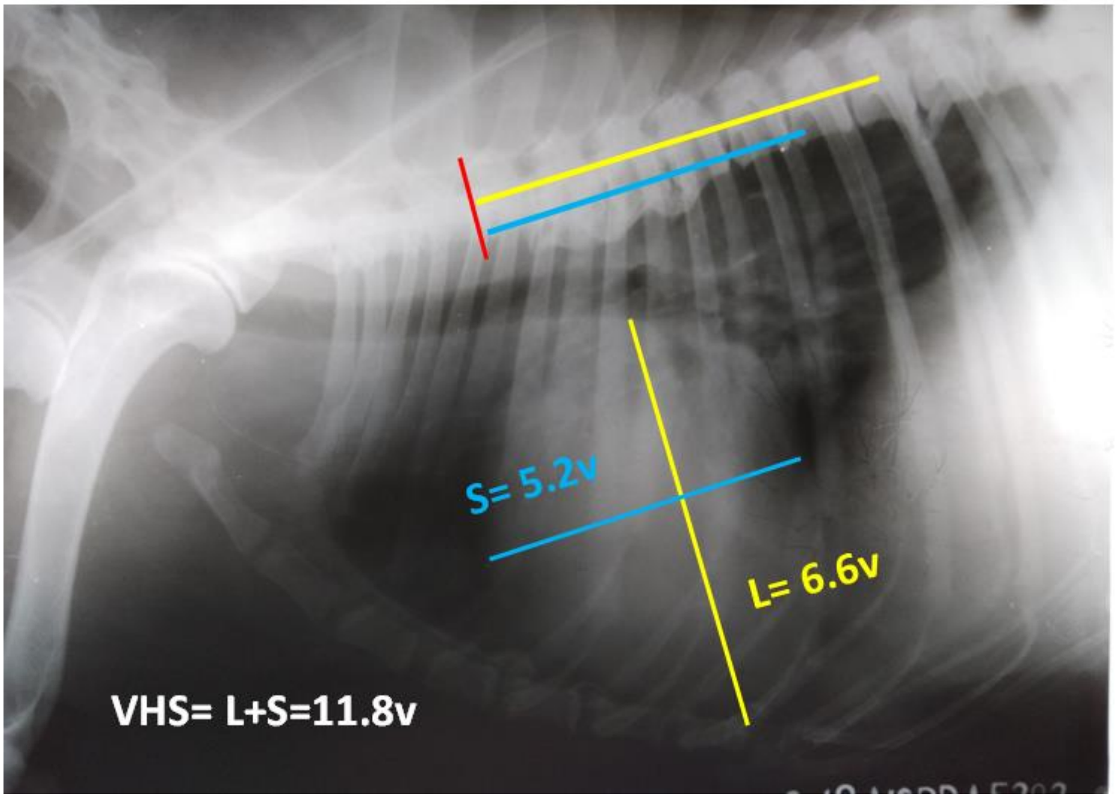


Plate 21 & 22: R-VHS and L-VHS of dog 6 (Group A) respectively.



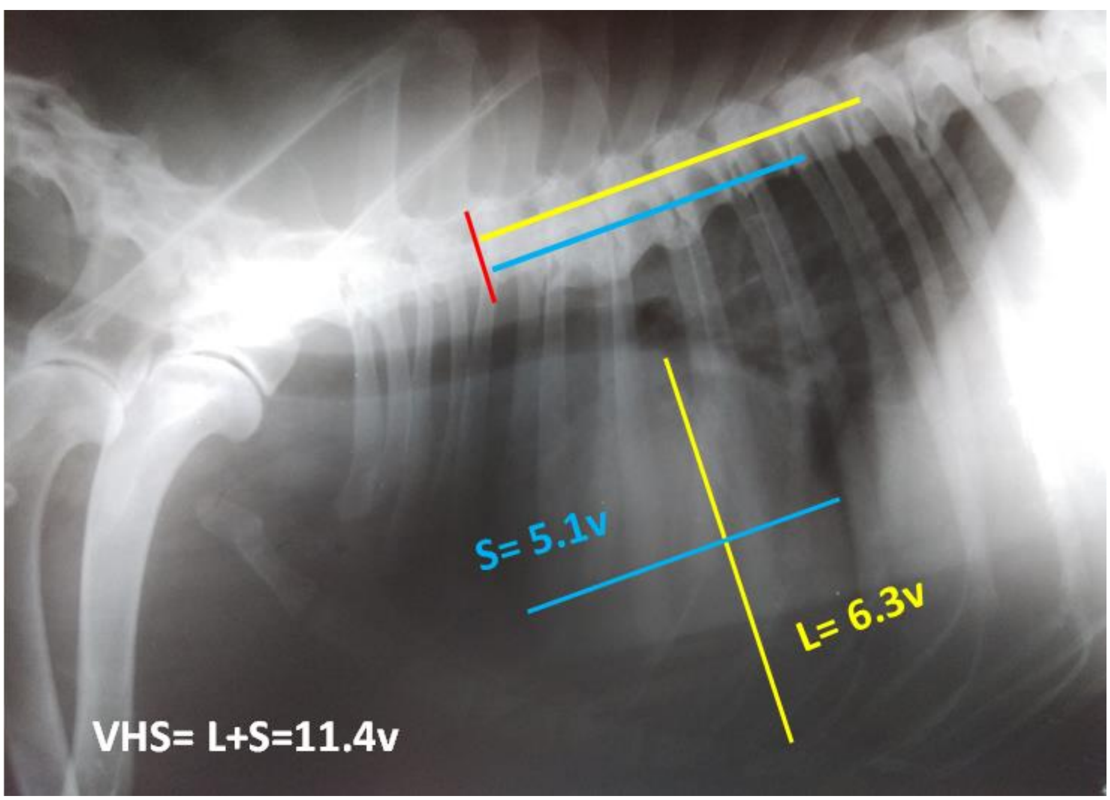
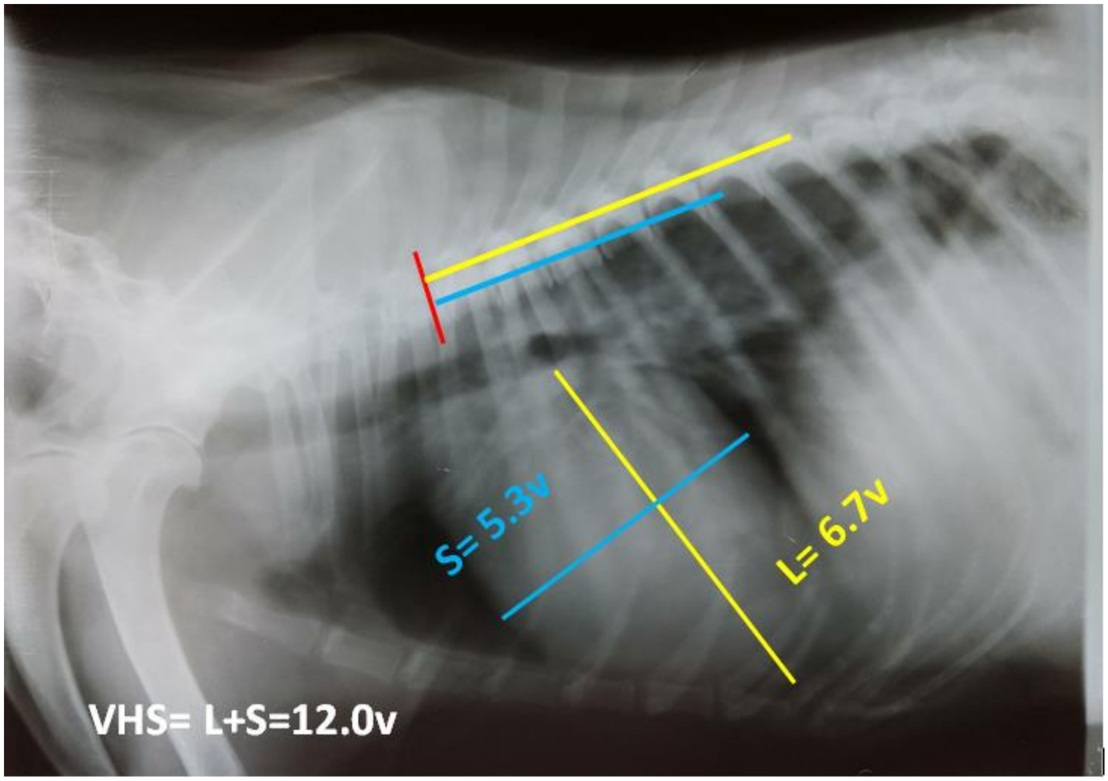


Plate 23 & 24: R-VHS and L-VHS of dog 1 (Group B) respectively.



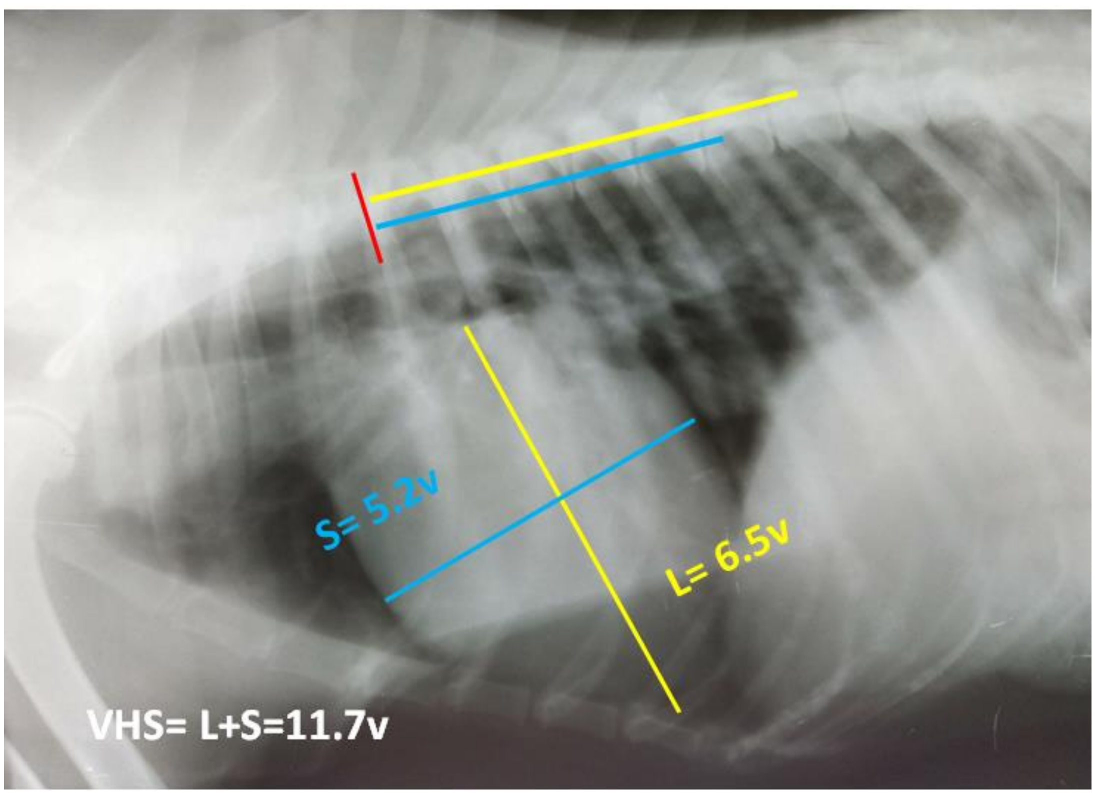
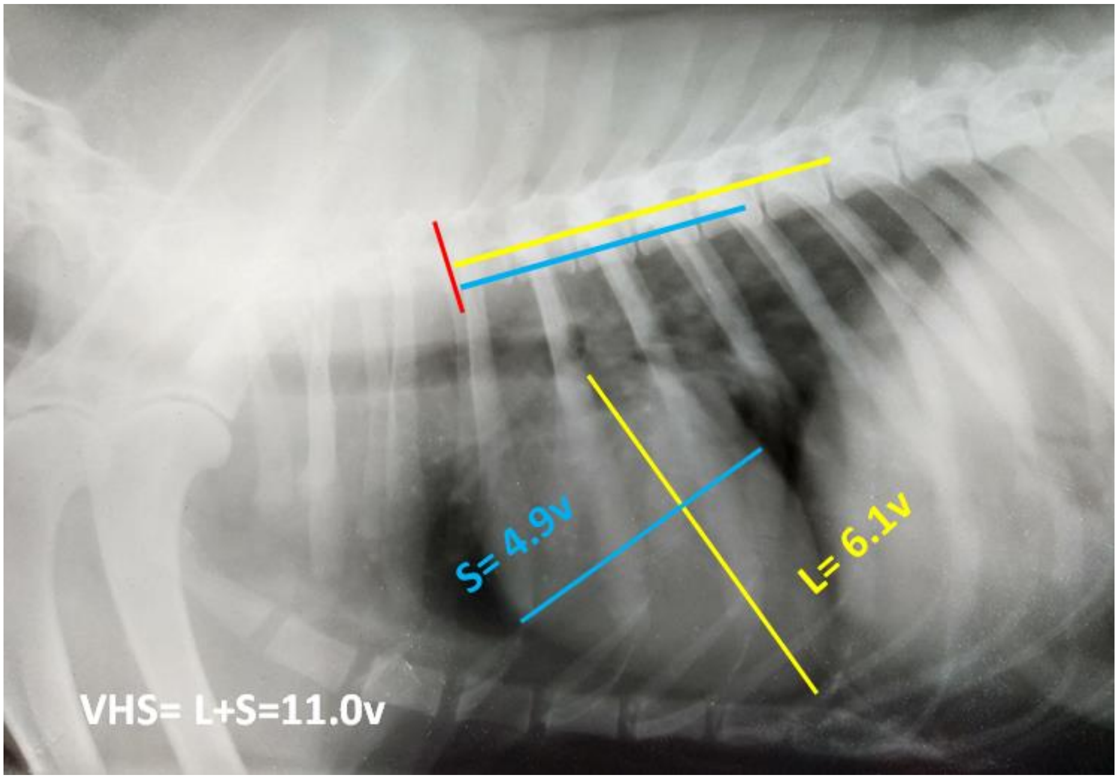


Plate 25 & 26: R-VHS and L-VHS of dog 2 (Group B) respectively.



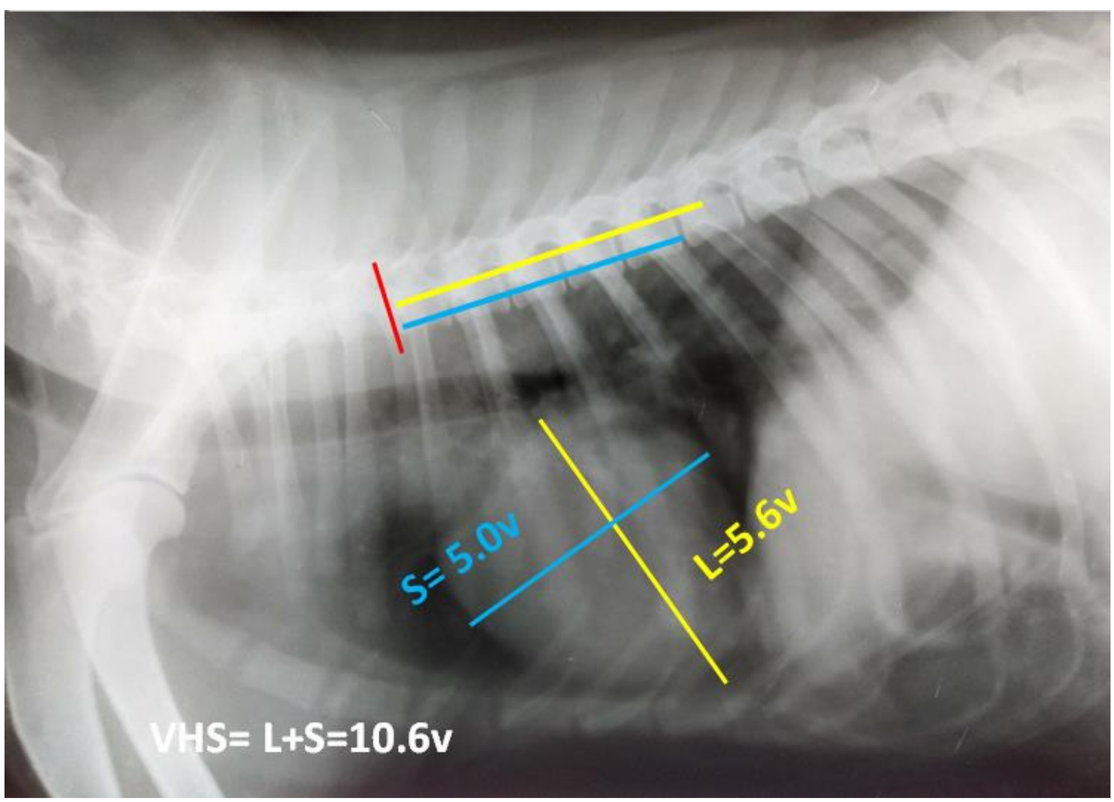
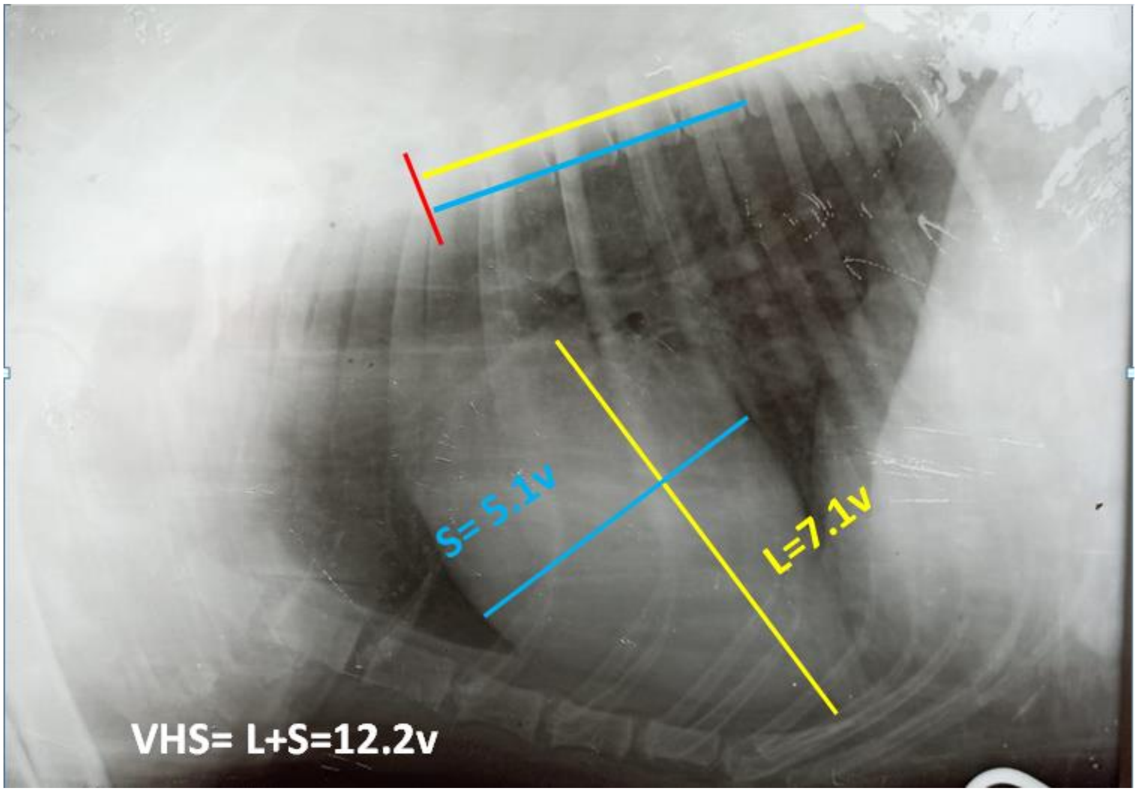


Plate 27 & 28: R-VHS and L-VHS of dog 3 (Group B) respectively.



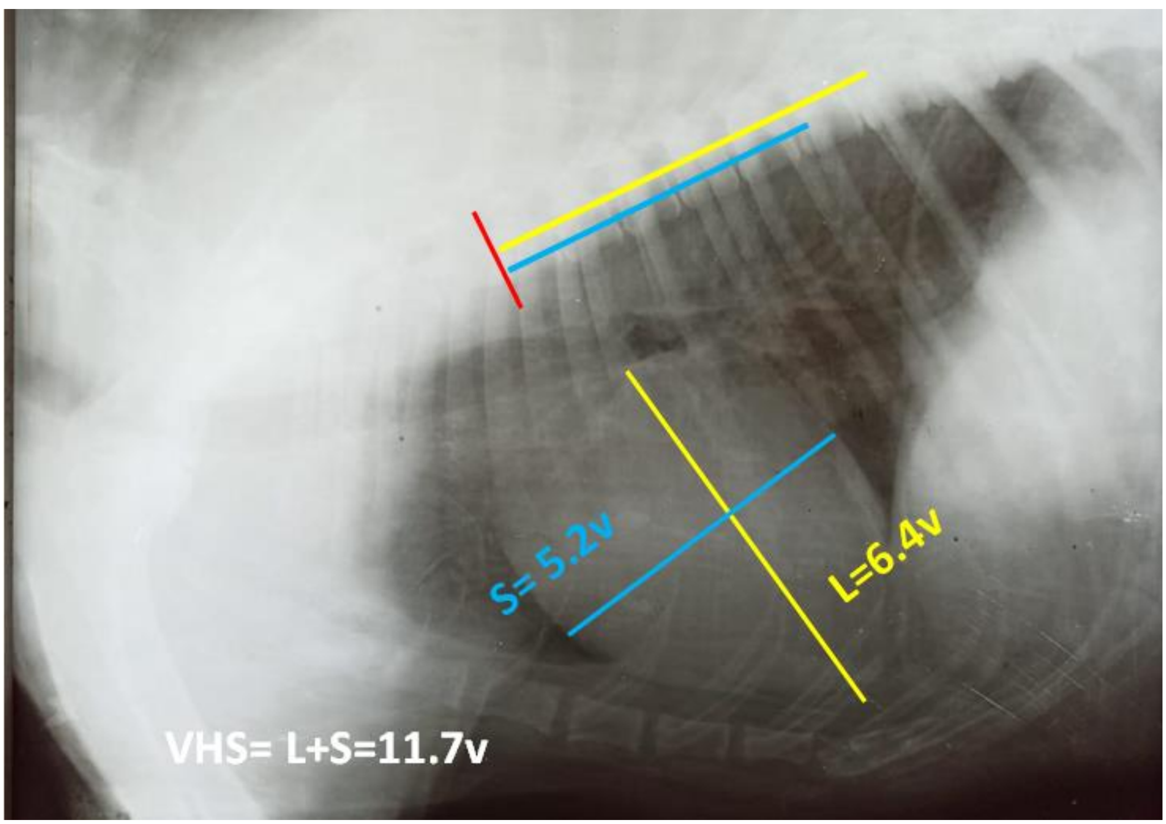
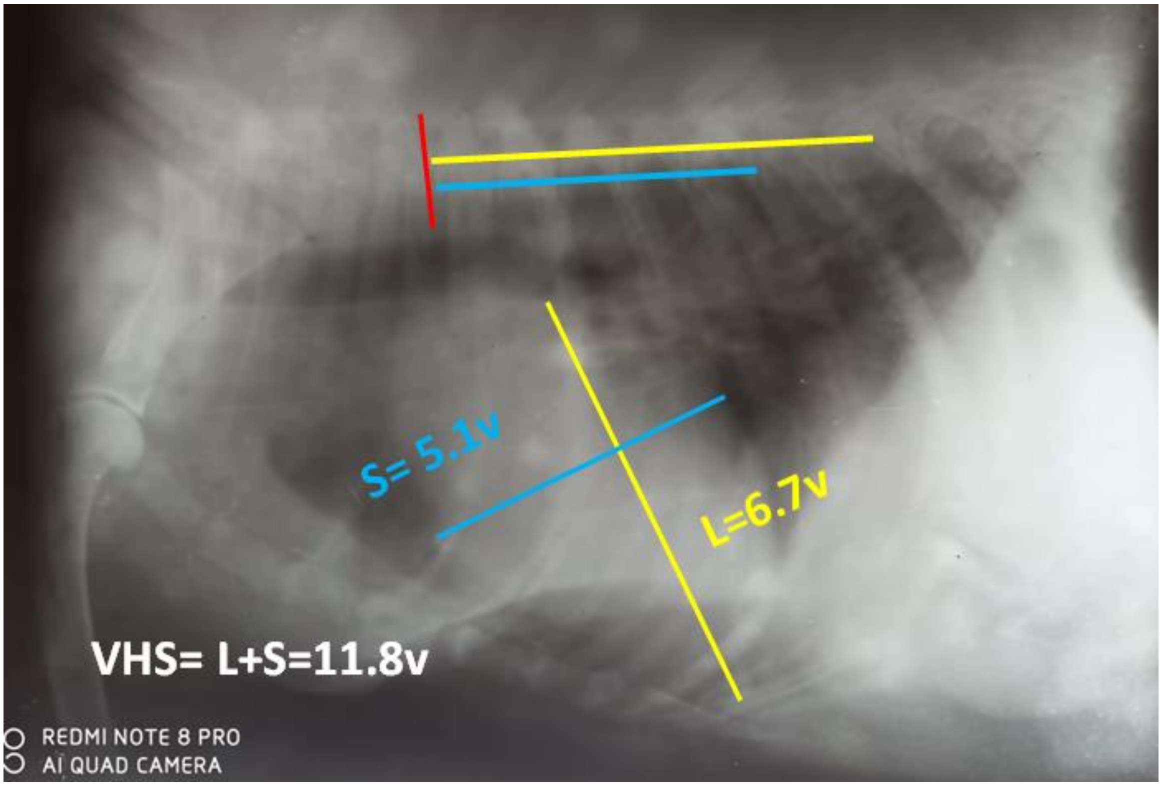


Plate 29 & 30: R-VHS and L-VHS of dog 4 (Group B) respectively.



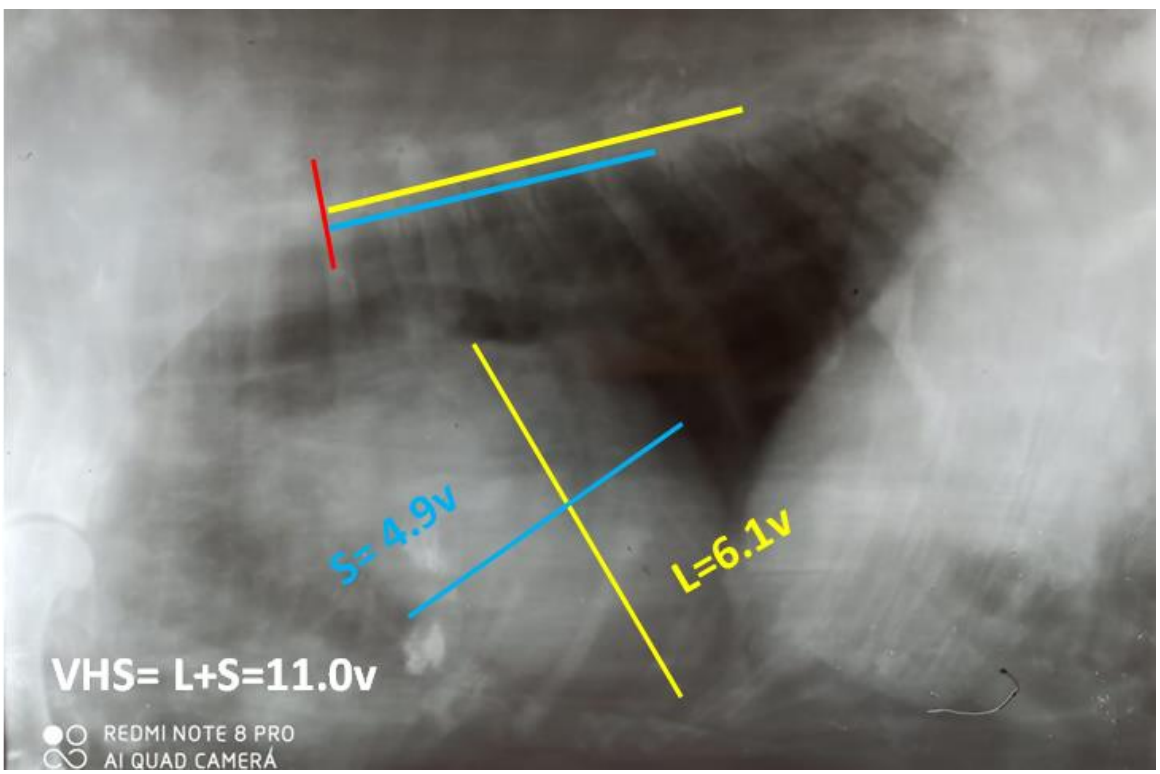
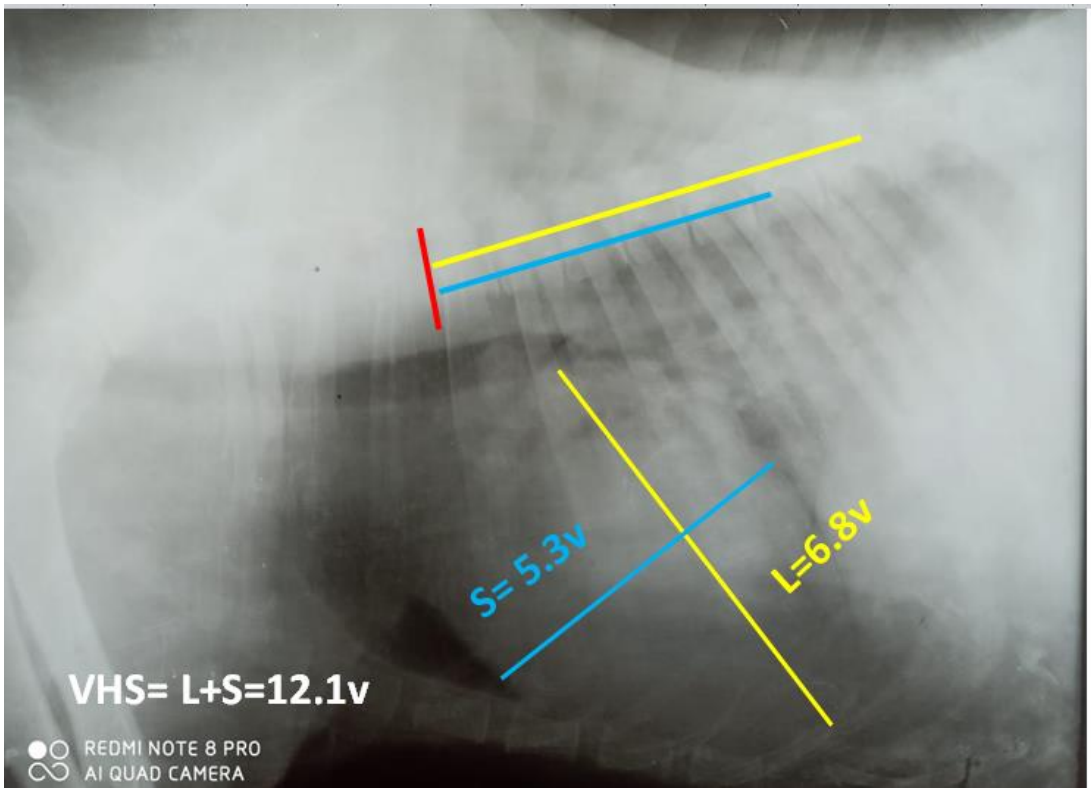


Plate 31 & 32: R-VHS and L-VHS of dog 5 (Group B) respectively.



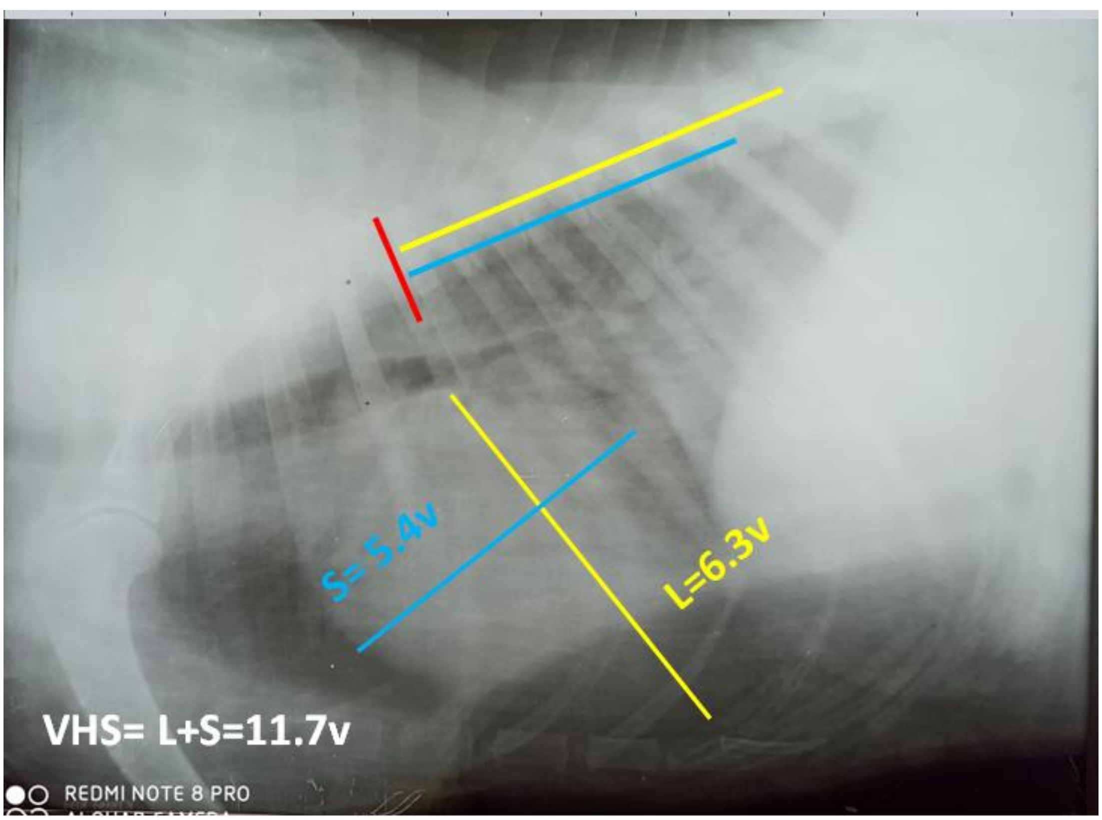


Plate 33 & 34: R-VHS and L-VHS of dog 6 (Group B) respectively.

The variations in the Mean ± SE values of VHS in Group A dogs on both right and left lateral thoracic radiograph were statistically significant (P value ≤ 0.05). The R-VHS and L-VHS of group A dogs was depicted in **Fig. 17.**

4.3.6.2.2 Vertebral Heart Score evaluation in Group B dogs

The Mean ± SE values of VHS in Group B dogs on right lateral thoracic radiograph was 11.8 ± 0.17v, which ranged between 11 to 12.2v and the same on left lateral thoracic radiograph was 11.3 ± 0.18v, which ranged between 10.6 to 11.7v.

The variations in the Mean ± SE values of VHS in Group B dogs on both right and left lateral thoracic radiograph were statistically significant (P value ≤ 0.05). The R-VHS and L-VHS of group B dogs was depicted in **Fig. 18.**

4.3.6.2.3 Comparative effect of right and left lateral recumbency on VHS

The Vertebral Heart Score values of both Group A and B dogs on right and left lateral thoracic radiography were depicted in **Table 11.**

4.3.6.2.3.1 Long Axis (L) of right lateral thoracic radiograph

The Mean ± SE values of long axis of right lateral thoracic radiograph in Group A dogs was 5.7 ± 0.04v, which ranged between 5.7 to 5.9v and that of Group B was 6.67 ± 0.13v, which ranged between 6.1 to 7.1v.

The variations in Mean ± SE values of long axis on right lateral thoracic radiographs of Group B dogs were increased significantly as compared to Group A dogs (P value ≤ 0.05) **(Fig.19)**

Fig. 17: Column showing Vertebral Heart Score values in Group A dogs on right and left lateral thoracic radiographs.

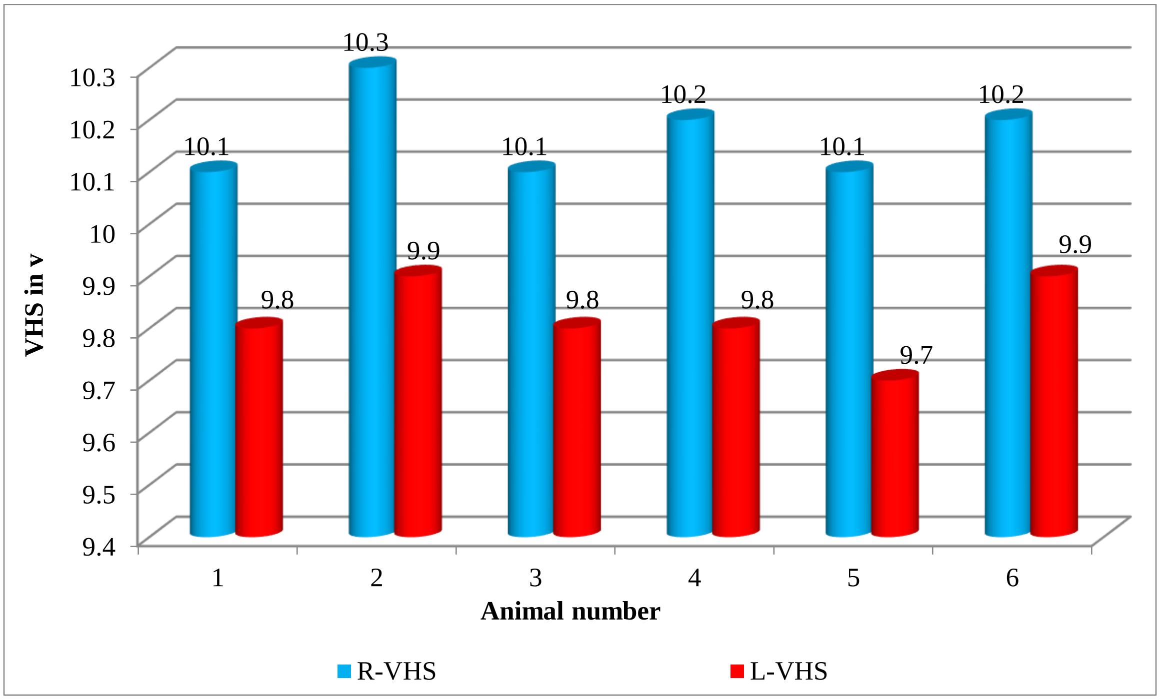
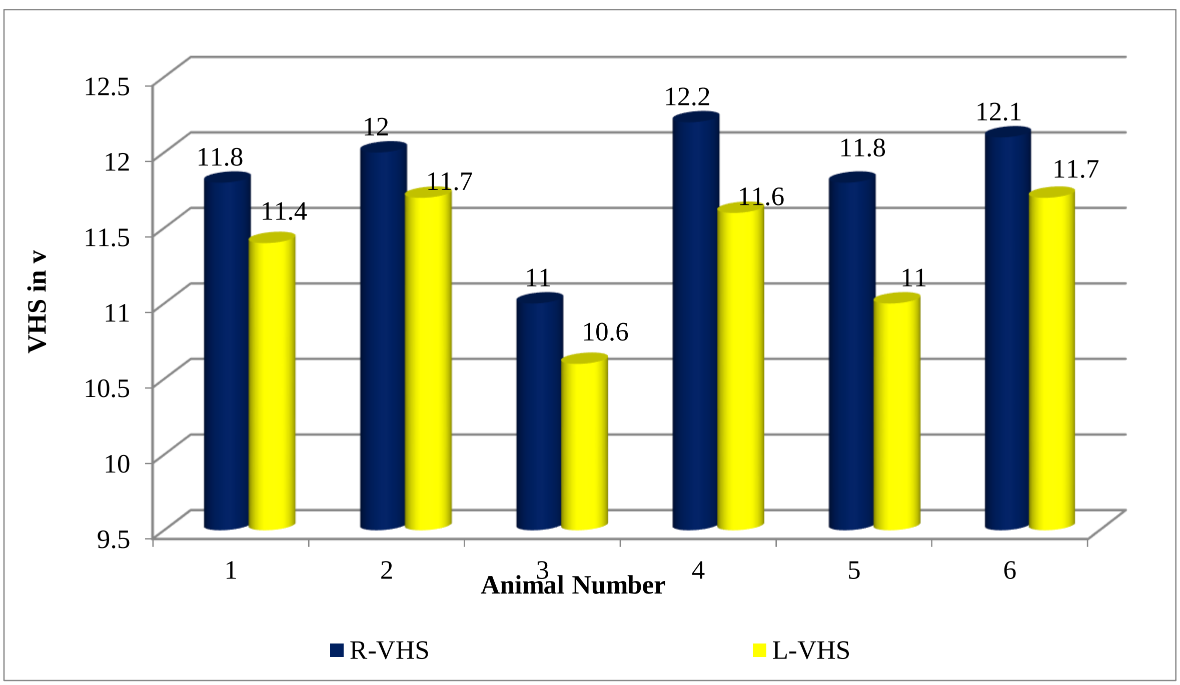


Fig. 18: Column showing Vertebral Heart Score values in Group B dogs on both right and left lateral thoracic radiographs



4.3.6.2.3.2 Short Axis (S) of right lateral thoracic radiograph

The Mean ± SE values of short axis of right lateral thoracic radiograph in Group A dogs was 4.38 ± 0.07v, which ranged between 4.2 to 4.6v and that of Group B was 5.15 ± 0.06v, which ranged between 4.9 to 5.3v.

The variations in Mean ± SE values of short axis on right lateral thoracic radiographs of Group B dogs were increased significantly as compared to Group A dogs (P value ≤ 0.05) **(Fig.20).**

4.3.6.2.3.3 Long Axis (L) of left lateral thoracic radiograph

The Mean ± SE values of long axis of left lateral thoracic radiograph in Group A dogs was 5.4 ± 0.08v, which ranged between 5.2 to 5.7v and that of Group B was 6.2 ± 0.13v, which ranged between 5.6 to 6.5.

The variations in Mean ± SE values of long axis on left lateral thoracic radiographs of Group B dogs were increased significantly as compared to Group A dogs (P value ≤ 0.05) **(Fig. 21).**

4.3.6.2.3.3 Short Axis (S) of left lateral thoracic radiograph

The Mean ± SE values of short axis of left lateral thoracic radiograph in Group A dogs was 4.33 ± 0.1v, which ranged between 4 to 4.6v and that of Group B was 5.13 ± 0.07v, which ranged between 4.9 to 5.4v.

The variations in Mean ± SE values of short axis on left lateral thoracic radiographs of Group B dogs were increased significantly as compared to Group A dogs (P value ≤ 0.05) **(Fig. 22).**

4.3.6.2.3.4 Long Axis (L) of right lateral VHS versus left lateral VHS

The variations in Mean ± SE values of long axis on right lateral thoracic radiographs were significantly greater than the same on left lateral thoracic radiographs both within and between the groups (P value ≤ 0.05) **(Fig. 23).**

4.3.6.2.3.5 Short Axis (S) of right lateral VHS versus left lateral VHS

The variations in Mean ± SE values of short axis on right and left lateral thoracic radiographs were statistically non-significant in both Group A and B dogs (P value ≥ 0.05). But these values varied significantly between the groups (P value ≤ 0.05) **(Fig. 24).**

4.3.6.2.3.6 Right lateral VHS versus left lateral VHS

The variations in the Mean ± SE values of VHS in Group A and B dogs on both right and left lateral thoracic radiograph were statistically significant (P value ≤ 0.05) **(Fig. 25).**

Table 11: The Mean ± SE values of VHS parameters in both Group A and B dogs on right and left lateral thoracic radiographs

Physiological parameters	Group A		Group B		P value between the groups
	R-VHS	L-VHS	R-VHS	L-VHS	
Long Axis (L) in v	5.7±0.04	5.4±0.08	6.67±0.13	6.2±0.13	0.0009*[R] 0.001*[L]
P value within the groups	0.007*		0.001*		
Short Axis (S) in v	4.38±0.07	4.33±0.1	5.15±0.06	5.13±0.07	0.00009*[R] 0.0001*[L]
P value within the groups	0.54		0.77		
Vertebral Heart Score (VHS) in v	10.1±0.03	9.8±0.03	11.8±0.17	11.3±0.18	0.00003*[R] 0.00009*[L]
P value within the groups	0.00001*		0.001*		

*represents statistical significance

[R] represents P value between R-VHS

[L] represents P value between L-VHS

Fig. 19: Column showing the values of Long Axis (L) in both Group A and B dogs on right lateral thoracic radiograph

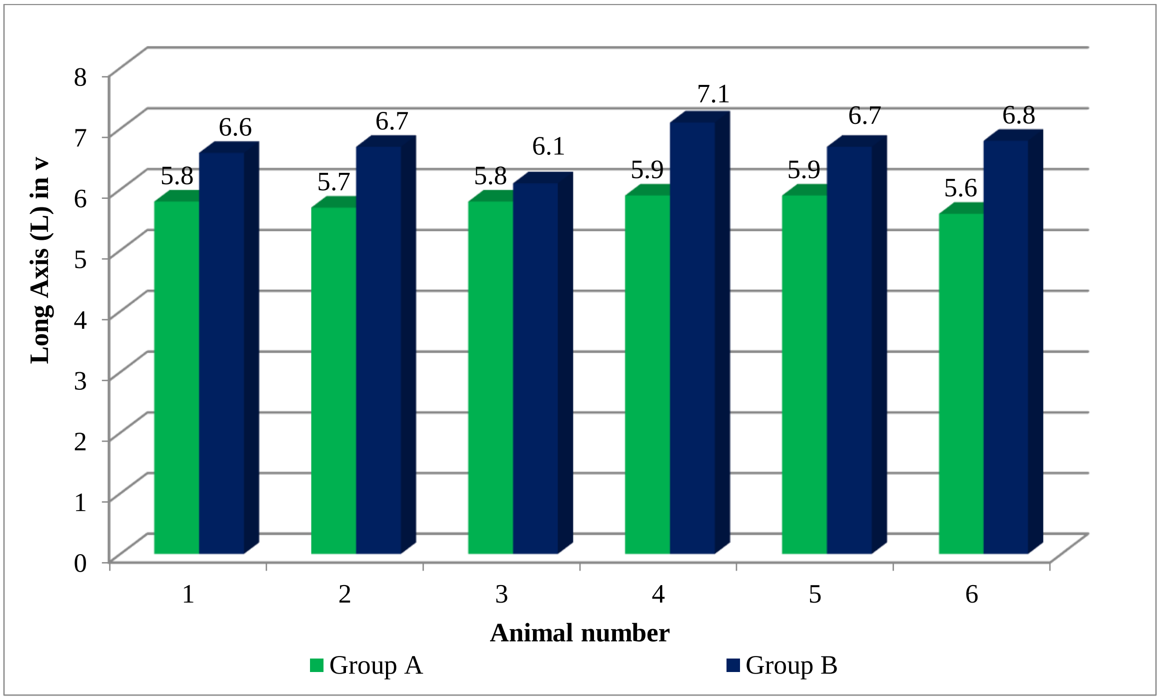


Fig. 20: Column showing the values of Short Axis (S) in both Group A and B dogs on right lateral thoracic radiograph

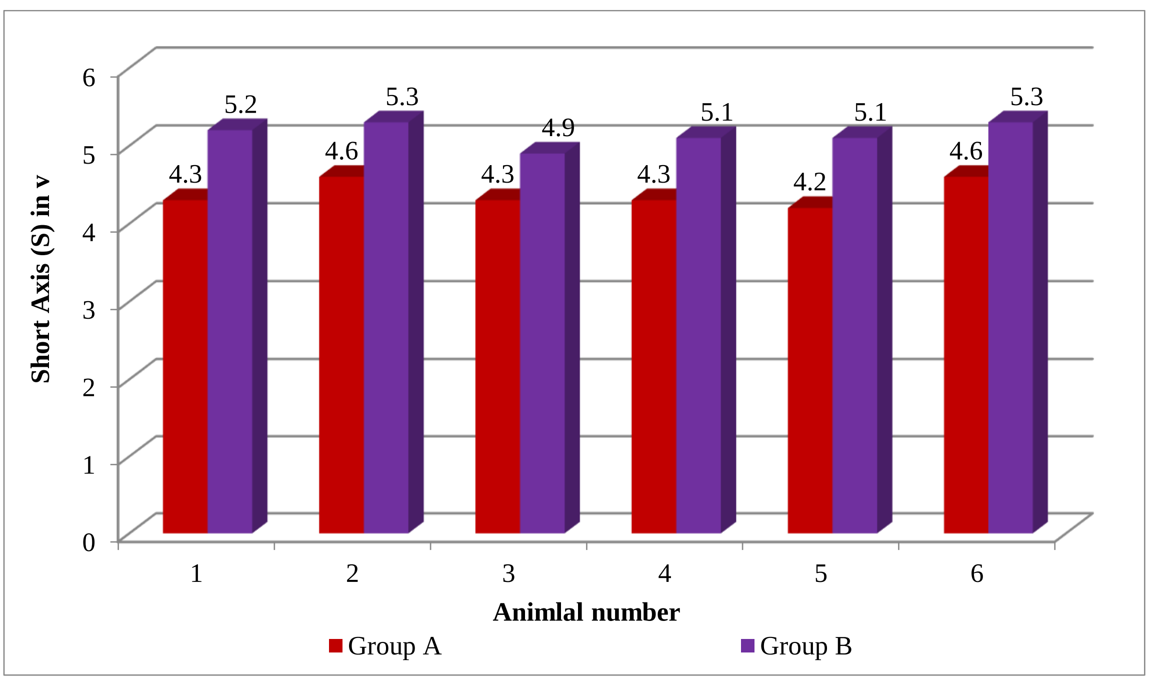


Fig. 21: Column showing the values of Long Axis (L) in both Group A and B dogs on left lateral thoracic radiograph

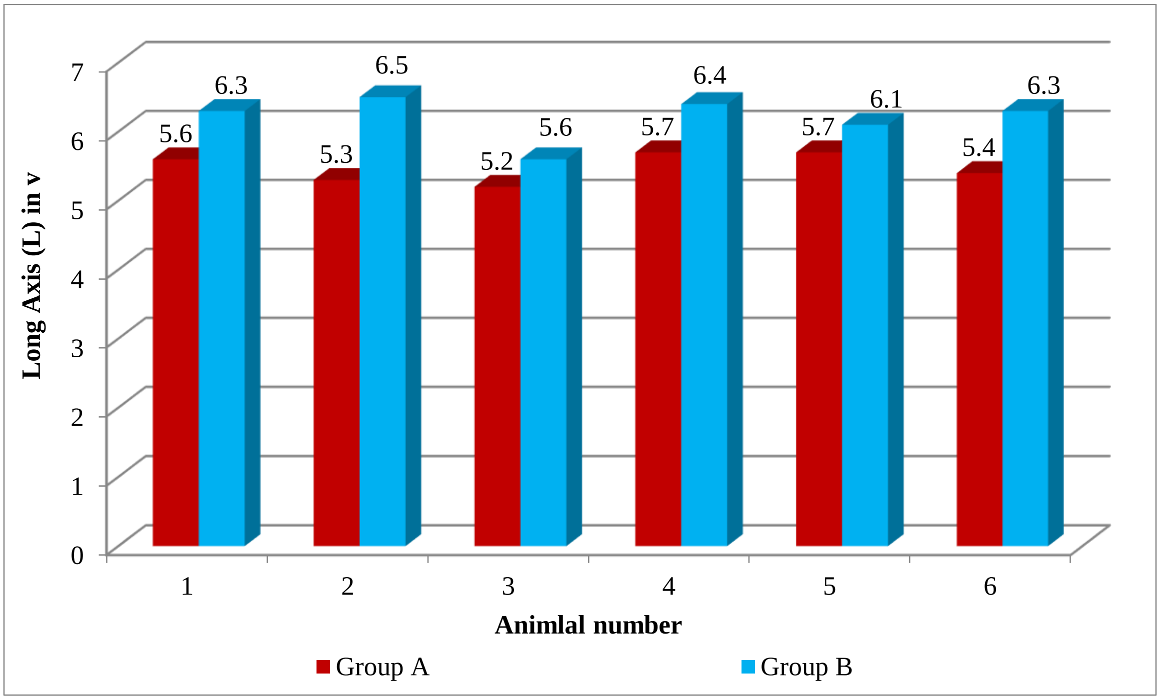


Fig. 22: Column showing the values of Short Axis (S) in both Group A and B dogs on left lateral thoracic radiograph

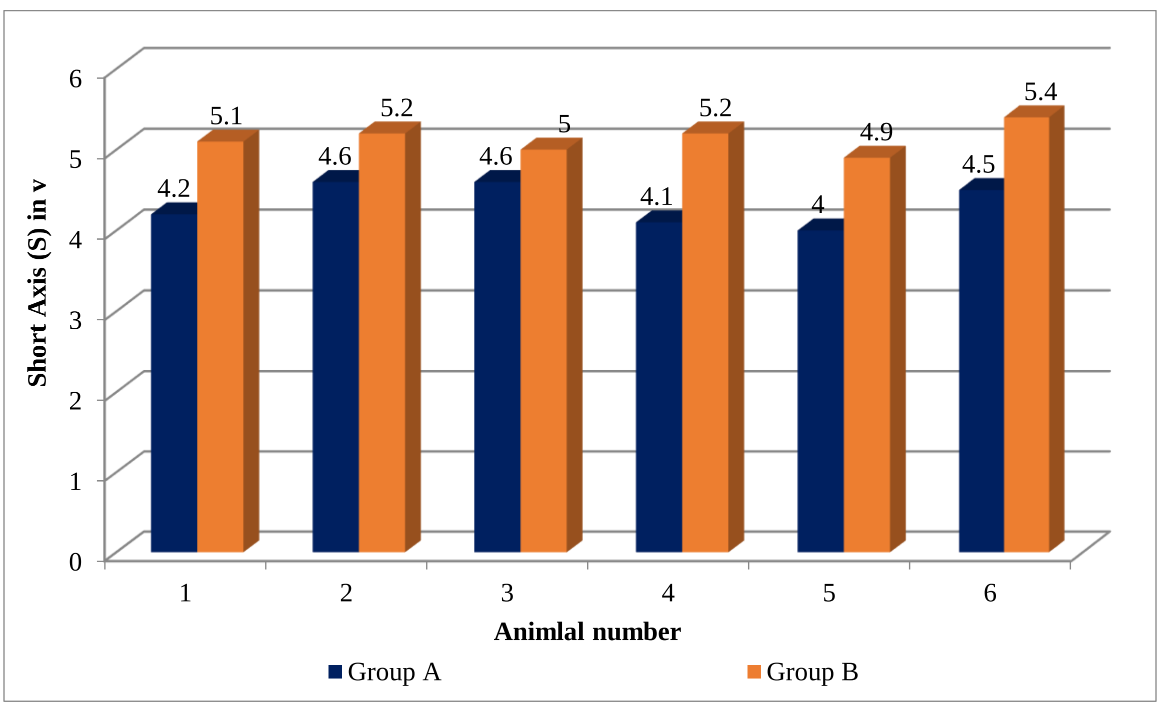


Fig. 23: Column showing the Mean ± SE values of long axis in both Group A and B dogs on right and left lateral thoracic radiograph

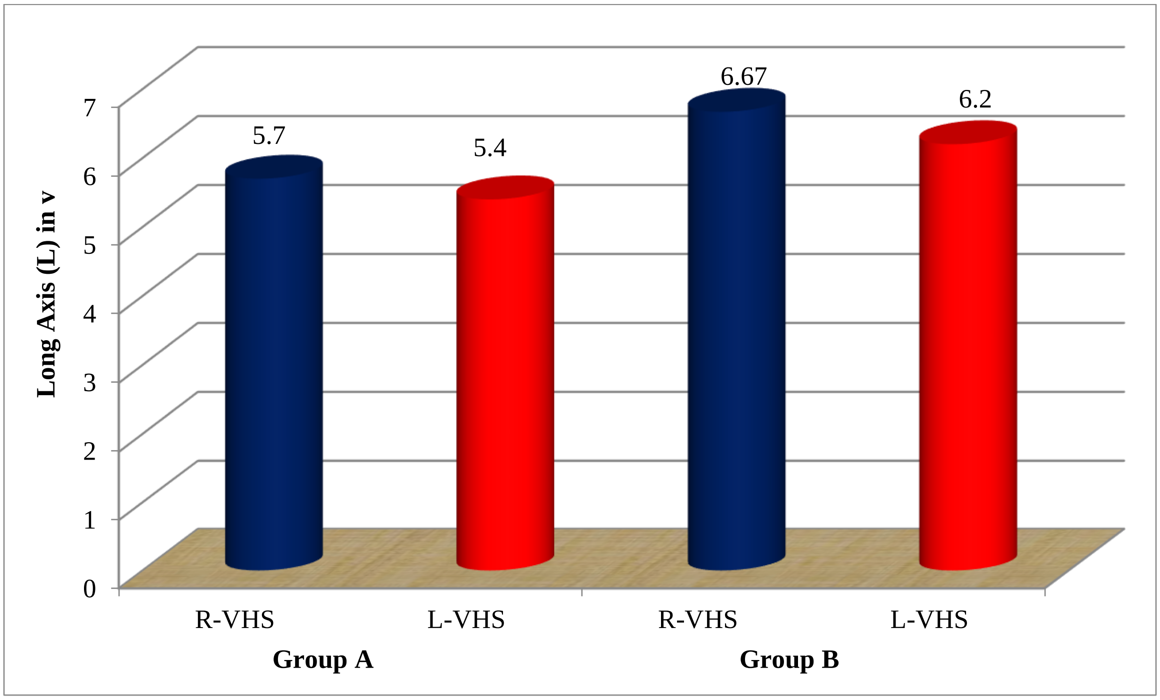


Fig. 24: Column showing the Mean ± SE values of short axis in both Group A and B dogs on right and left lateral thoracic radiograph

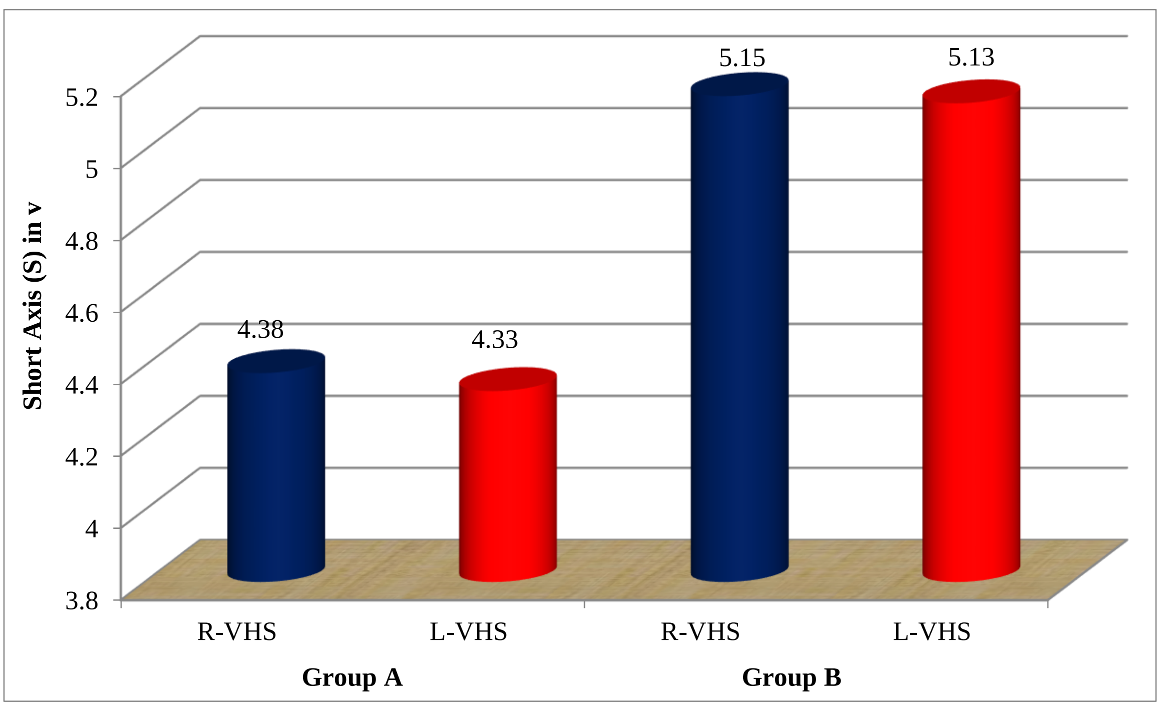
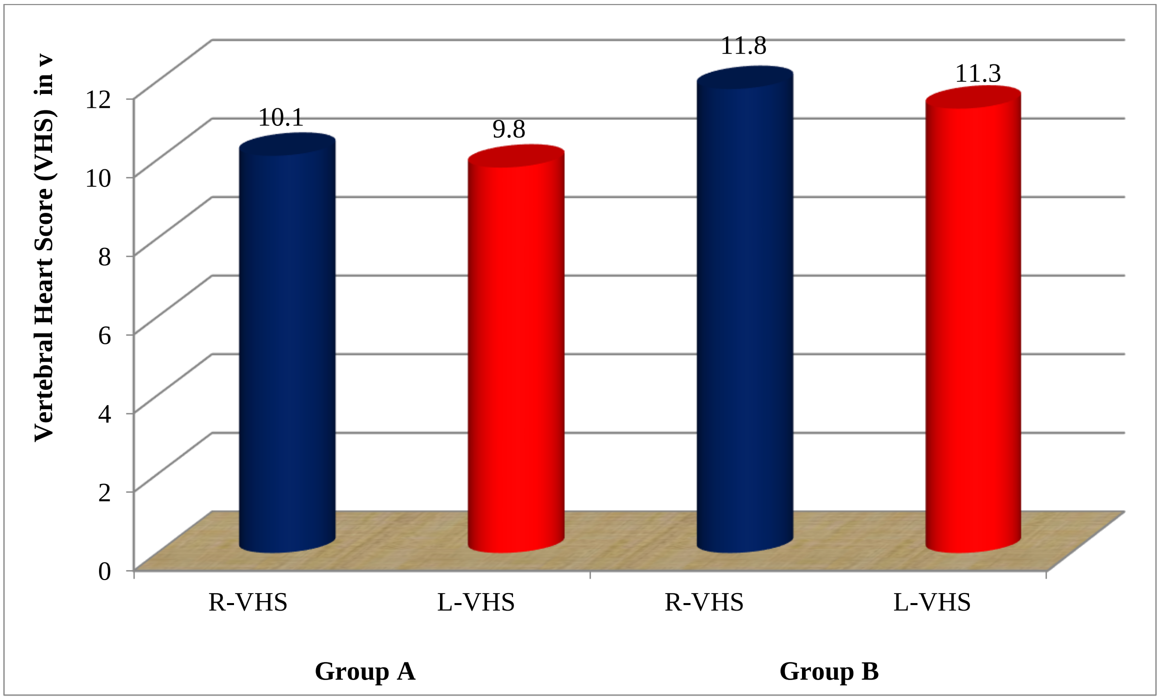


Fig. 25: Column showing the Mean ± SE values of VHS in both Group A and B dogs on right and left lateral thoracic radiograph



4.3.6.3 Electrocardiography (ECG)

The mean ± SE values of electrocardiographic parameters of Group A dogs were P wave amplitude (0.2 ± 0.06 mV), Q wave amplitude (0.45 ± 0.01 mV), R wave amplitude (1.75 ± 0.04 mV), S wave amplitude (0.17 ± 0.007 mV), T wave amplitude (0.36 ± 0.015 mV), PR interval (0.08 ± 0.03 seconds), QT interval (0.2 ± 0.07 seconds), QRS width (0.04 ± 0.003 seconds), P wave duration (0.04 ± 0.0007 seconds) and the same for Group B dogs were P wave amplitude (0.2 ± 0.01 mV), Q wave amplitude (0.41 ± 0.017 mV), R wave amplitude (1.36 ± 0.20 mV), S wave amplitude (0.23 ± 0.03 mV), T wave amplitude (0.45 ± 0.067 mV), PR interval (0.07 ± 0.003 seconds), QT interval (0.195 ± 0.007 seconds), QRS width (0.05±0.004 seconds), P wave duration (0.04 ± 0.003 seconds).

The mean ± SE values of electrocardiographic parameters were within normal reference range for given body weight in Group A Labrador retriever dogs and the variations between Group A and B dogs were statistically non-significant (P value ≥ 0.05) **(Table 12).**

The electrocardiogram (ECG) of group A dogs was shown in **Plate 35 and 36,** whereas for group B dogs was shown from **Plate 37 to 42.**

Table 12: Mean ± SE values of ECG parameters in both Group A & B dogs (n=6)

ECG waves and complexes	Group A	Group B	P value
P wave amplitude (mV)	0.2 ± 0.06 (0.19-0.23)	0.2 ± 0.01 (0.18-0.25)	0.74
Q wave amplitude (mV)	0.45 ± 0.01 (0.39-0.5)	0.41 ± 0.017 (0.38-0.5)	0.15
R wave amplitude (mV)	1.75 ± 0.04 (1.6-1.9)	1.36 ± 0.20 (0.7-1.9)	0.1
S wave amplitude (mV)	0.17 ± 0.007 (0.14-0.19)	0.23 ± 0.03 (0.15-0.36)	0.15
T wave amplitude (mV)	0.36 ± 0.015 (0.32-0.41)	0.45 ± 0.067 (0.33-0.78)	0.26
PR interval (seconds)	0.08 ± 0.03 (0.07-0.092)	0.07 ± 0.003 (0.069-0.09)	0.31
QT interval (seconds)	0.2 ± 0.07 (0.19-0.24)	0.195 ± 0.007 (0.17-0.22)	0.37
QRS width (seconds)	0.04 ± 0.003 (0.04-0.05)	0.05±0.004 (0.04-0.06)	0.09
P wave duration (seconds)	0.04 ± 0.0007 (0.037-0.042)	0.04 ± 0.003 (0.038-0.06)	0.21

Note: The variations in all the above parameters were statistically non-significant

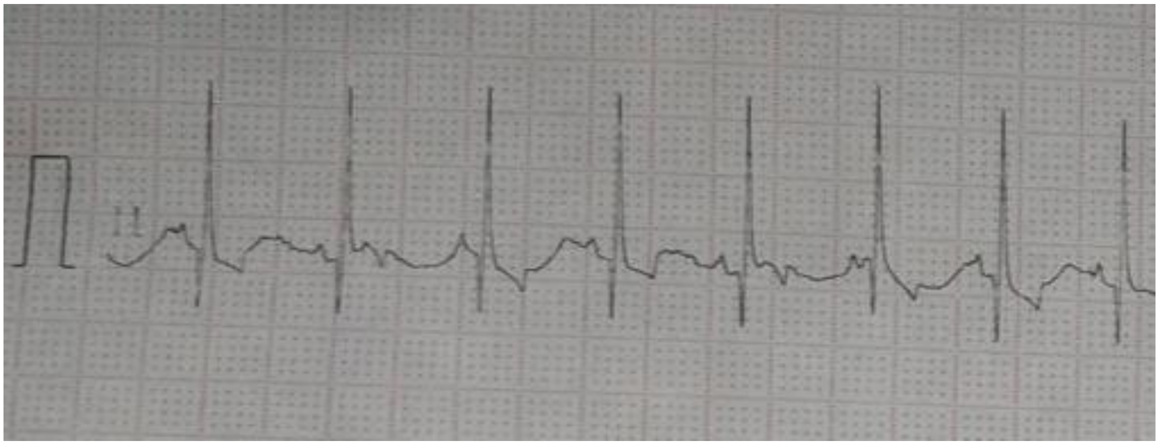


Plate 35: ECG of a healthy Labrador retriever dog (Group A) showing normal rhythm, rate and measurements of different ECG waves and complexes.

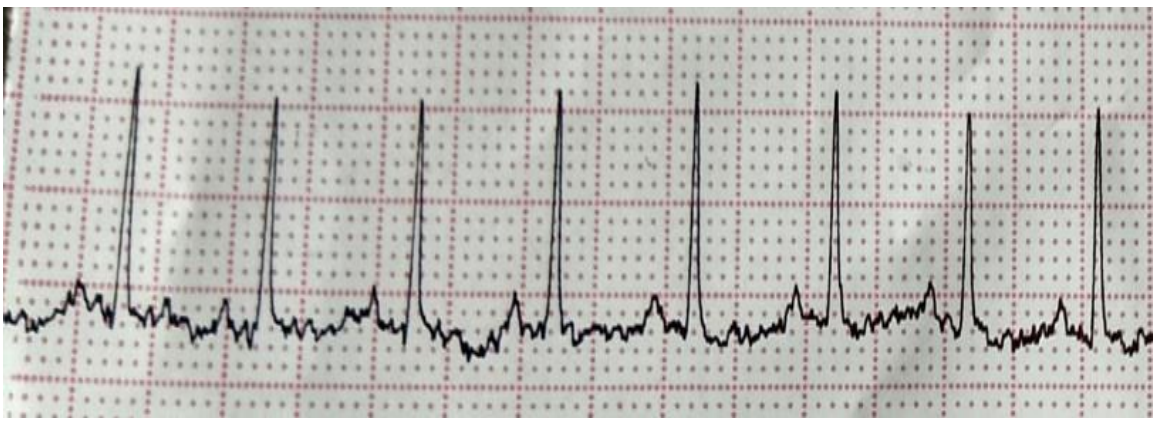


Plate 36: ECG of a healthy Labrador retriever dog (Group A) showing normal rhythm, rate and demarcation for measurements of different ECG waves and complexes with electrical alternans.

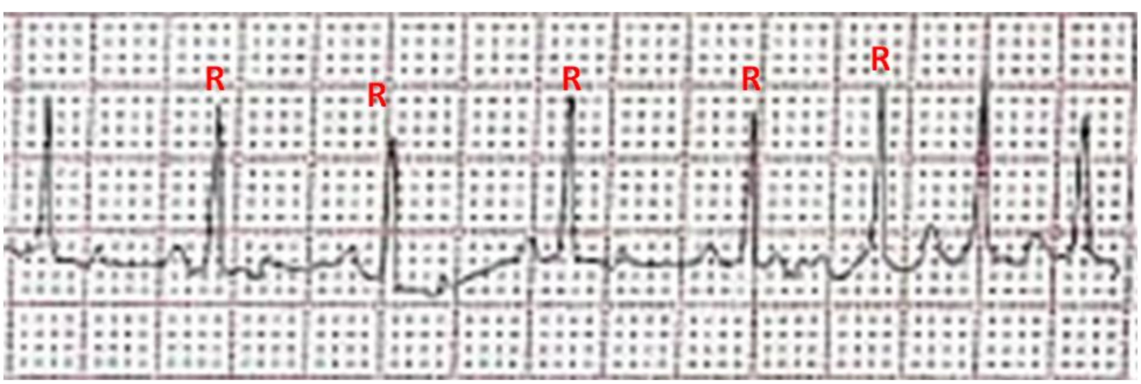


Plate 37: ECG (Group B1 dog) showing irregular R wave interval suggestive of sinus arrhythmia and right ventricular enlargement.

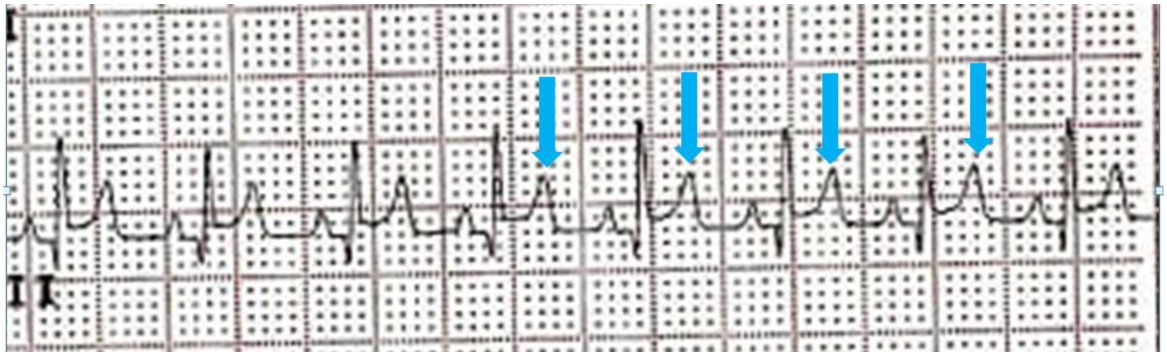


Plate 38: ECG (Group B2 dog) showing ST elevation or increased T wave amplitude (blue arrow) suggestive of early sign of myocardial infarction or myocardial oedema, coronary artery occlusion or may be due to elevation in the movement of diaphragm during respiration.

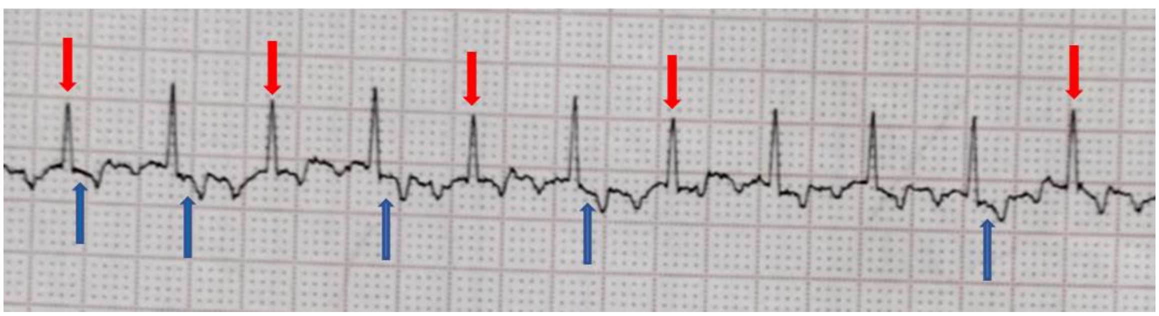


Plate 39: ECG (Group B3 dog) showing ST depression or S dip (blue arrow) and short decreased R wave amplitude (red arrow) suggestive of myocardial infarction, pericardial or pleural effusion, hypovolemia, hypothermia, hypothyroidism, obesity and right ventricular enlargement.

Plate 40: ECG (Group B4 dog) showing decreased QRS width suggestive of myocardial hypoxia and left ventricular enlargement.

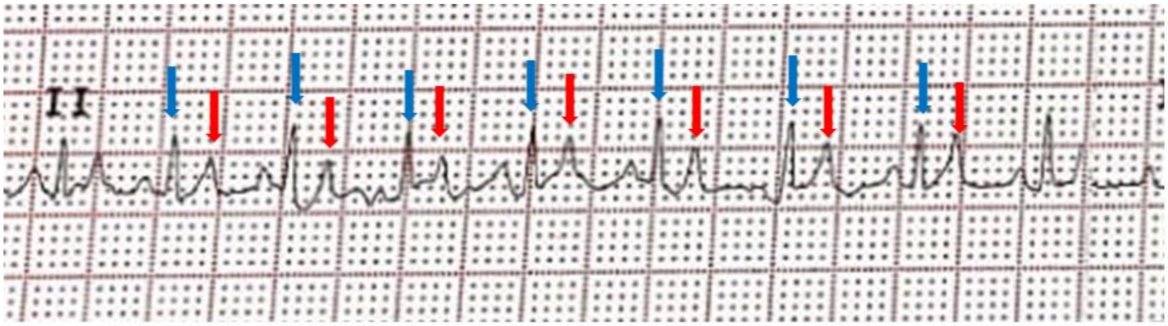


Plate 41: ECG (Group B5 dog) showing ST elevation or increased T wave amplitude (red arrow) suggestive of early sign of myocardial infarction or myocardial oedema, coronary artery occlusion or may be due to elevation in the movement of diaphragm during respiration and short R waves (blue arrow) indicative of myocardial infarction, pericardial or pleural effusion, hypovolemia, hypothermia, hypothyroidism, obesity, electrolyte imbalance and left ventricular enlargement.

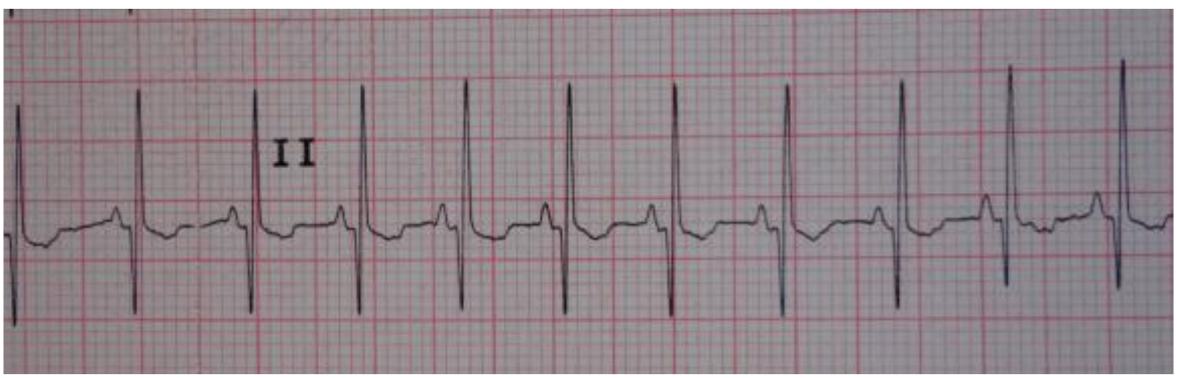


Plate 42: ECG (Group B6 dog) showing Q dip / increased Q wave amplitude suggestive of right ventricular enlargement.

4.3.6.4 Echocardiography

The mean ± SE values of M mode echocardiographic parameters of Group A dogs were LVIDd (3.04 ± 0.38cm), LVIDs (1.97 ± 0.33cm), IVSd (1.01 ± 0.16cm), IVSs (1.18 ± 0.23cm), LVPWd (1.03 ± 0.14cm), LVPWs (1.23 ± 0.15cm), EDV (42.02 ± 10.1ml), ESV (15.93 ± 4.53ml), LA/Ao ratio (1.28 ± 0.06), FS (37.1 ± 6.42 %), EF (66.57 ± 5.95 %) and that of Group B dogs were LVIDd (5.17 ± 0.7cm), LVIDs (4.91 ± 0.38 cm), IVSd (0.72 ± 0.07 cm), IVSs (0.79 ± 0.09cm), LVPWd (0.83 ± 0.07cm), LVPWs (1.91 ± 0.88cm), EDV (100.83 ± 24.68ml), ESV (80.62 ± 20.6ml), LA/Ao ratio (1.56 ± 0.08), FS (17.33 ± 4.87 %) and EF (35.72 ± 9.4 %)

The mean ± SE values of echocardiographic parameters were within normal reference range for given body weight in Group A Labrador retriever dogs and the variations between Group A and B dogs were statistically significant (P value $\leq$ 0.05) except for IVSd, IVSs, LVPWd and LVPWs (Pvalue $\geq$ 0.05) **(Table 13).**

The 2D and M-mode echocardiograms of Group A dogs were shown in **Plate 43 and 44,** whereas echocardiogram of the various cardiac diseases of Group B dogs was shown from **Plate 45 to 51.**

4.3.6.5 Correlation of VHS findings with ECG and echocardiography

The Group A dogs with normal cardiac silhouette in thoracic radiography had normal ECG and echocardiographic parameters within the reference range of Labrador retriever breed of dogs and revealed normal cardiac functioning on 2D and M-mode echocardiography with VHS of 10.1 ± 0.03v and 9.8 ± 0.03v on right and left lateral recumbency respectively.

Table 13: Mean ± SE values of echocardiographic parameters in both group dogs

Echocardiographic parameters	Group A	Group B	P value
LVIDd (cm)	3.04 ± 0.38 (1.51-3.78)	5.17 ± 0.7 (1.87-6.5)	0.02*
LVIDs (cm)	1.97 ± 0.33 (0.79-2.6)	4.91 ± 0.38 (3.08-5.62)	0.001*
IVSd (cm)	1.01 ± 0.16 (0.42-1.45)	0.72 ± 0.07 (0.35-0.85)	0.14
IVSs (cm)	1.18 ± 0.23 (0.48-1.81)	0.79 ± 0.09 (0.62-1.19)	0.14
LVPWd (cm)	1.03 ± 0.14 (0.42-1.51)	0.83 ± 0.07 (0.53-1.04)	0.25
LVPWs (cm)	1.23 ± 0.15 (0.73-1.75)	1.91 ± 0.88 (0.79-6.33)	0.46
EDV (ml)	42.02 ± 10.1 (6.17-68.3)	100.83±24.68 (24.57-196.95)	0.05*
ESV (ml)	15.93 ± 4.53 (1.07-24.58)	80.62 ± 20.6 (5.42-144.36)	0.01*
LA/Ao ratio	1.28 ± 0.06 (1.07-1.41)	1.56 ± 0.08 (1.33-1.82)	0.02*
FS (%)	37.1 ± 6.42 (28-69.05)	17.33 ± 4.87 (4.44-39.8)	0.03*
EF (%)	66.57 ± 5.95 (57.35-95.4)	35.72 ± 9.4 (9.97-77.9)	0.02*

* represents the statistical significance between the groups

The Group B dogs showed various cardiac diseases with radiographic cardiomegaly of different heart chambers, had altered ECG and echocardiographic parameters. Echocardiography showed marked dilation of ventricles, altered systolic functions and one dog showed thickened and fibrotic mitral valve suggestive of MMVD with increased VHS values i.e., 11.8 ± 0.17v and 11.3 ± 0.18v on right and left lateral recumbency respectively.

Therefore the variations in the VHS values were subjectively correlated with electrocardiogram (ECG) and echocardiographic findings in both groups.

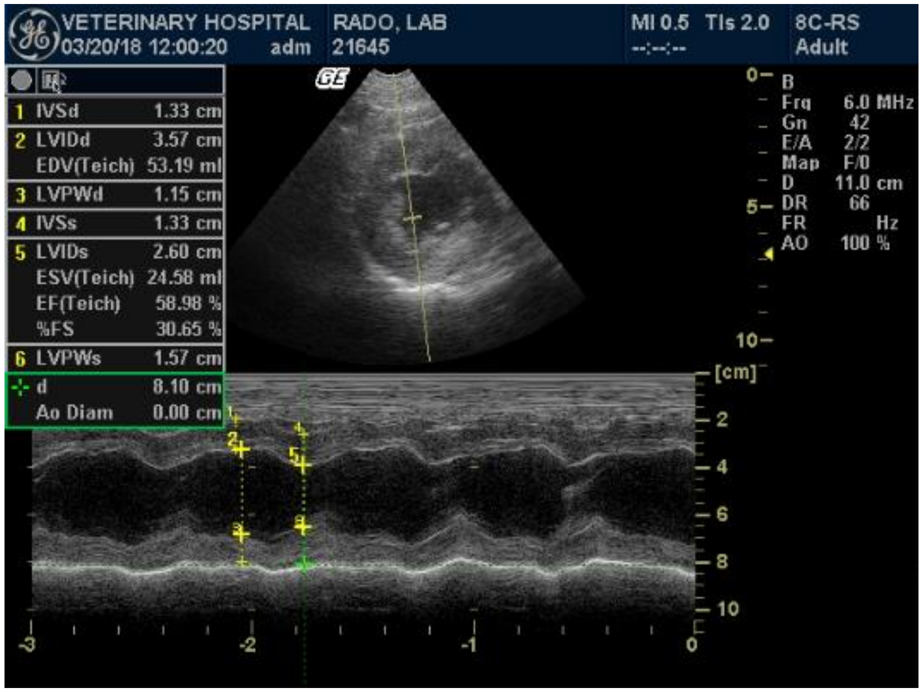


Plate 43: Right parasternal long axis M mode echocardiographic view of a normal Labrador retriever dog.

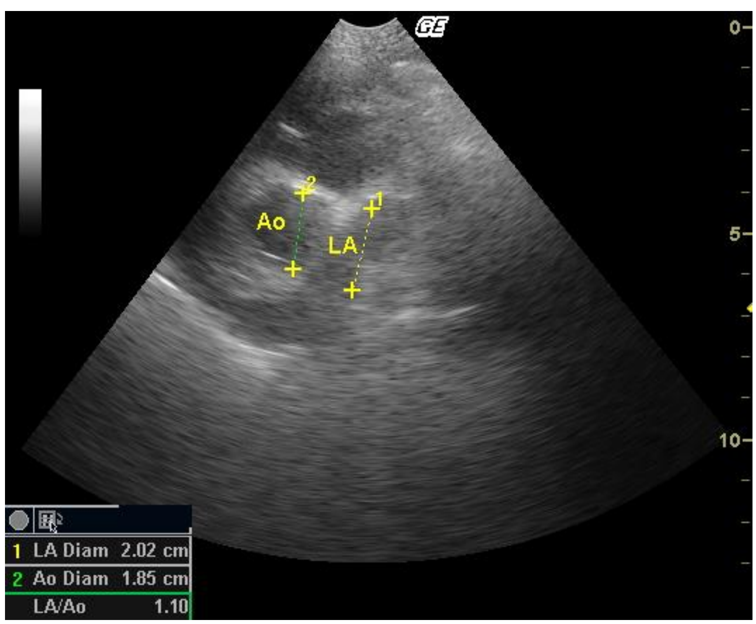


Plate 44: Right parasternal transverse echocardiographic view showing normal LA/Ao ratio of Group A Labrador retriever dog.

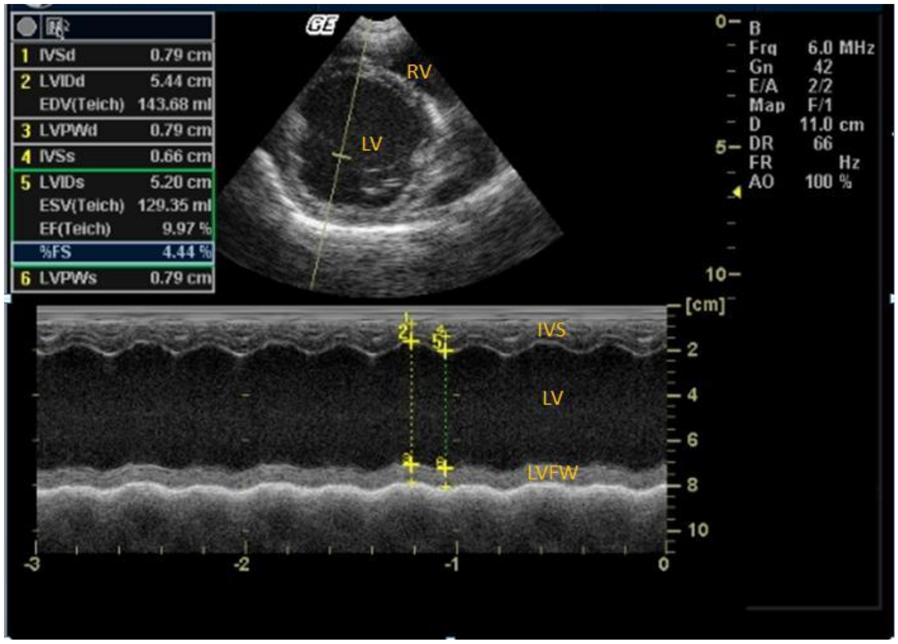


Plate 45: M mode echocardiographic view showing left ventricular enlargement indicative of dilated cardiomyopathy (DCM).

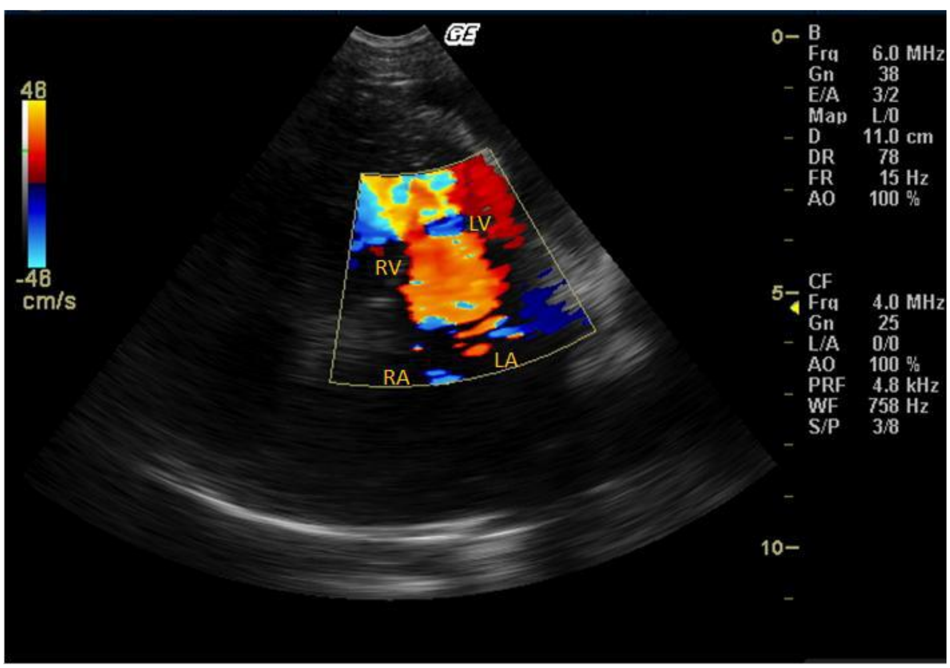


Plate 46: Colour flow doppler echocardiographic view showing thickened mitral valve and regurgitation of blood indicative of mitral valve degeneration (MVD).

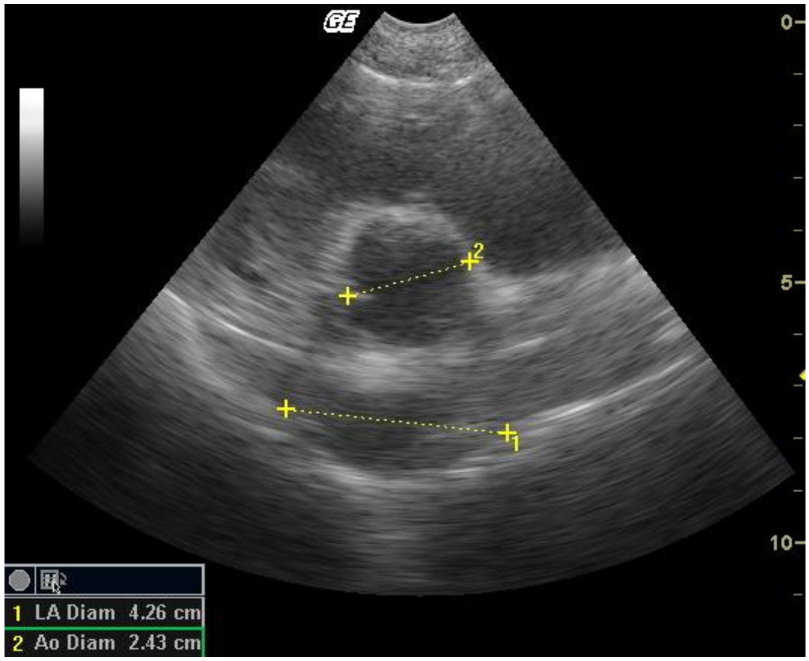


Plate 47: Right parasternal long axis echocardiographic view showing increased LA/Ao ratio *i.e.*, 1.75 (> 1.5) indicative of left atrial enlargement.

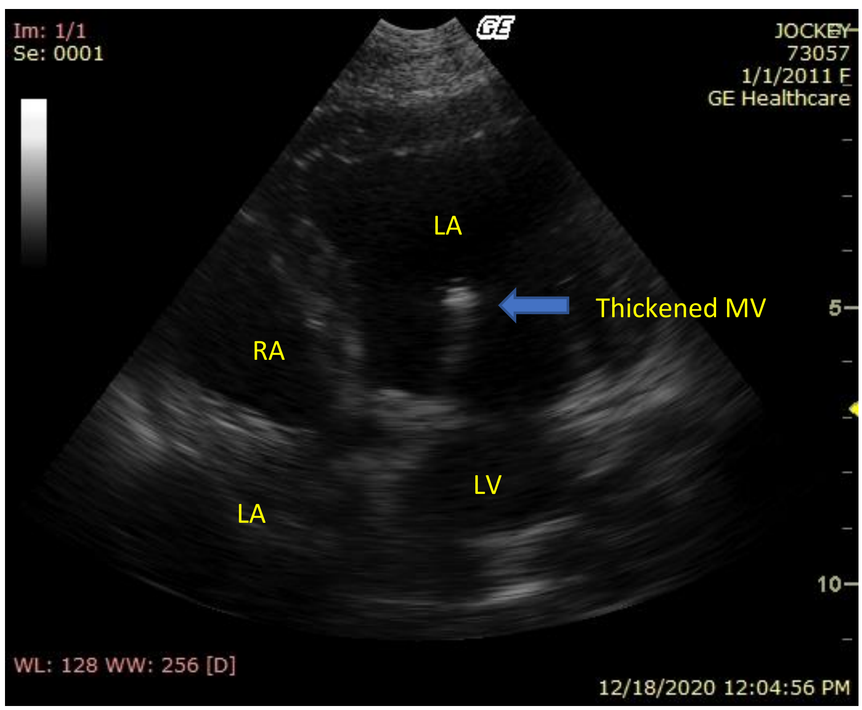


Plate 48: Left parasternal long axis echocardiographic view showing thickened mitral valve (blue arrow) indicative of Myxomatous Mitral Valve Degeneration (MMVD).

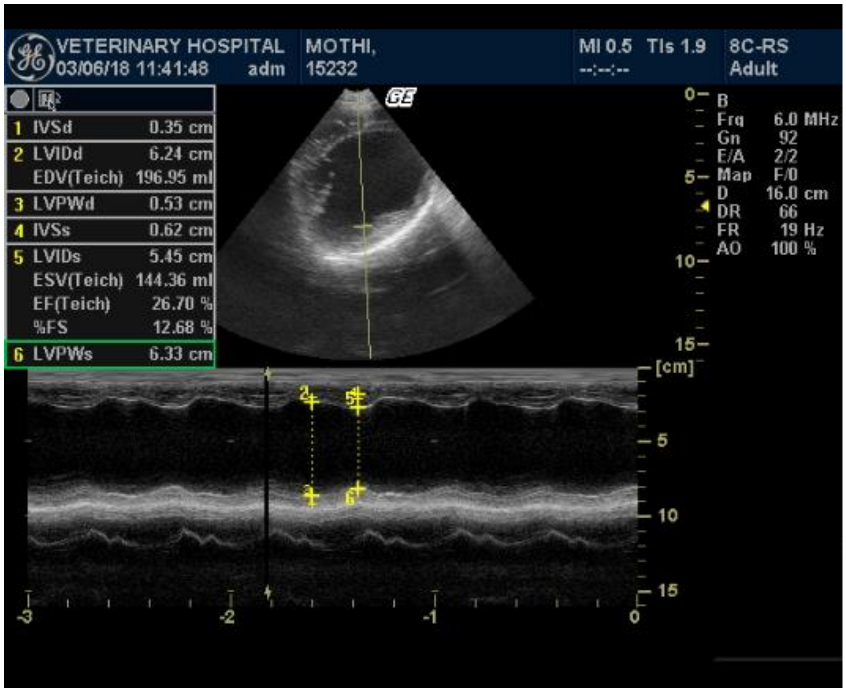


Plate 49: Right parasternal long axis echocardiographic view showing marked reduction in left ventricular systolic function, EF and FS indicative of left ventricular enlargement.

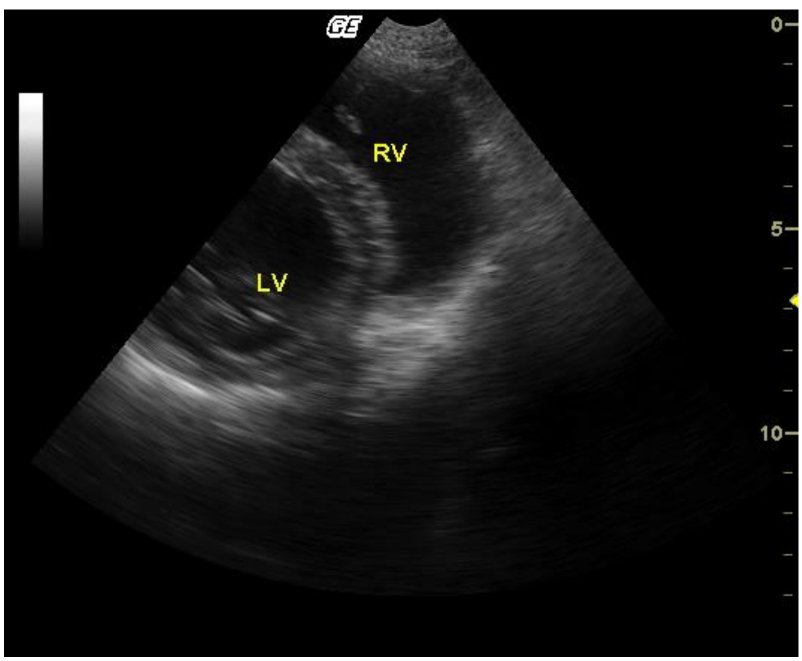


Plate 50: Right parasternal long axis echocardiographic view showing right and left ventricular enlargement (biventricular enlargement).

Discussion

V. DISCUSSION

The present study was carried out to compare the influence of right and left lateral thoracic radiography on VHS in Labrador retriever breed of dogs. The results were discussed as follows,

5.1 Occurrence of cardiac diseases in dogs

In the present study, 1.94% (165 out of 8472) of the dogs presented to the Veterinary College Hospital, Hebbal, Bengaluru during June 2017 to May 2018 were diagnosed with cardiac diseases.

The similar observations on cardiac diseases were observed by Detweiler *et al.* (1968), who reported incidence of cardiac disorders in dogs to an extent of 1%; Deepti (2005) 1.17%; Haritha *et al.* (2017) 1.77%; Himalini *et al.* (2017) 1.61% and Badsar *et al.* (2018) 2.41%.

5.1.1 Breed-wise occurrence

The occurrence of cardiac disease in Labrador retriever breed of dogs was 24.84%, accounted for the highest breed affected with cardiac diseases followed by Mongrel (14.54%; 24/165), Pomeranian (11.51%; 19/165), Golden retriever (9.69%; 16/165), Pug (9.09%; 15/165), German shepherd (6.66%; 11/165), Boxer (6.06%; 10/165), Great Dane (3.63%; 6/165) and Rottweiler (3.03%; 5/165).

Similar findings were observed by Soares *et al.* (2010), who reported that the cardiac diseases were most common in large and giant breeds; Rajkumar (2013) reported the highest occurrence of cardiac diseases in Labrador retriever (27.2%); Deepti (2015) reported the highest occurrence of CHF in Labrador retriever (25%); Himalini *et al.* (2017) reported the highest prevalence in Labrador retriever (44%) and lowest in Cocker spaniel, Saint bernese, Pug, Bullmastiff, Lhasa apso, Doberman *i.e.*, 4% each.

This pattern of variation in occurrence could be attributed to more number of Labrador retrievers and Mongrels in the geographical location.

5.1.2 Age-wise occurrence

The middle aged (4-8 years) dogs were over represented in both the groups, this could be due to increasing popularity of the Labrador breed in the geographical area where the study was conducted.

The highest occurrence of cardiac diseases was recorded in middle aged Labrador retriever breed. This pattern of occurrence of cardiac diseases in different age group was in concurrence with observations of Calvert *et al.* (1997); Guglielmini (2003); Martin *et al.* (2009); Wess *et al.* (2010) and Mourya *et al.* (2018) who reported higher incidence of cardiac diseases in middle aged dogs.

5.1.3 Gender-wise occurrence

The occurrence of cardiac diseases in Labrador retriever breed of dogs was highest in males as compared to females. This was in concurrence with findings of Detweiler and Patterson, (2006); Lombard (1984); Swenson *et al*. (1996); Calvert *et al*. (1997); Martin *et al.*, (2009); Rajkumar (2013); Haritha *et al.* (2017); Reetu *et al.* (2017)

and Hoque *et al.* (2019) who reported highest occurrence in males of different breeds due to preference of the pet owners for male dogs.

5.2 Diagnosis of cardiac diseases

5.2.1 Clinical signs and physical examination

All the dogs in the Group B showed one or more signs of cardiac diseases such as exercise intolerance, weakness, syncope, pale mucous membrane, weak femoral pulse, cough, dyspnoea, tachypnoea, ascites, tachycardia, arrhythmia, polydipsia, inappetance, weight loss and elevated body temperature. Two dogs in Group B showed severe dry coughing along with other signs of cardiac diseases which increases on exercise and were not responding to therapy. These findings were in agreement with Dukes-Mcewan *et al.* (2003).

There was severe cough in two dogs in the present study, where similar signs were observed by Atkins (1994) and Ristic (2004), who reported due to compression of left mainstream bronchus by large left atrium. The similar signs were also reported by Rajamohan (2018) in Labrador retriever dogs with DCM and Mourya *et al.* (2018) in dogs with cardiomegaly. Mounika *et al.* (2020) reported the exercise intolerance in obese dogs with cardiovascular diseases.

5.2.2 Clinical parameters

5.2.2.1 Body weight

The body weight of the Group B was significantly greater than that of Group A. This could be due to association of body weight and obesity to the cardiac diseases.

Borges *et al.* (2016) also reported obesity as one of the factor associated with heart disease in their study on associated risk factors of cardiovascular alteration in dogs. Thengchaisri *et al.* (2014) opined abdominal obesity rather than overall obesity was associated with heart diseases in dogs.

5.2.2.2 Body Condition Score (BCS)

The variations in mean values of BCS between the groups were statistically non-significant. This could be due to no association of BCS with cardiac disease occurrences. This was in agreement with the findings of Goutal *et al.* (2010) and Thengchaisri *et al.* (2014). However, the obesity increased the risk of development of hypertersion and negatively impacts cardiovascular and pulmonary functions predisposing them for cardiac diseases as stated by Kuruvilla and Frankel (2003).

5.2.3 Physiological parameters

5.2.3.1 Rectal temperature (°F)

The mean values of rectal temperature varied non-significantly between the groups. This was in agreement with findings of Ristic (2004), who reported that alteration in temperature is not a good indicator for cardiac disease. However Dillon and Brawner (1995) and Peddle *et al.* (2009) recorded a significant elevation in temperature in bacterial endocarditis and venacaval syndrome in dogs.

The radiographic procedure for VHS evaluation was carried out in a non-stressful environment and in conscious animals, where no sedatives and anaesthetics were used.

Hence there was no significant variations in temperature within both groups *i.e.,* before and after VHS evaluation.

5.2.3.2 Heart rate (beats per minute)

The significant elevation in heart rate was noticed in Group B dogs. The tachycardia might be due to ventricular dysfunction or excitation of the animal during examination. This was in agreement with Tidholm and Jonsson (1997); Dukes-McEwan *et al.* (2003), Schneider *et al.* (2011) and Kanno *et al.* (2016). This was further supported by the observations of Pagani *et al.* (1991), who noticed tachycardia and tachypnoea when the dogs were introduced into a new environment due to enhanced sympathetic activation, and a shift from a predominantly vagal tone to an increased sympathetic activity.

The adopted procedure resulted in statistically non-significant difference in heart rate within the groups *i.e.,* before and after VHS evaluation.

5.2.3.3 Respiratory rate (breaths per minute)

The mean values of respiratory rate varied significantly between the groups. Four dogs of Group B showed tachypnoea and three dogs showed dyspnoeic signs. This elevated respiratory rate in Group B dogs could be due to left heart failure causing pulmonary edema or right sided heart failure with ascites and/or progressive anaemia. This was in concurrence with Gomph (2007), Bomassi (2007) and Kanno *et al.* (2016). However, the tachypnoea in Group B dogs could be due to panting caused by excitation or introduction into the new environment as reported by Pagani *et al.* (1991).

The adopted procedure resulted in non-significant variation in the respiration rate within the groups *i.e.,* before and after VHS evaluation.

5.2.3.4 Blood pressure (mmHg)

The blood pressure in all the dogs was measured in closed environment to avoid alteration in the readings from blood pressure monitor. The significant elevation in systolic blood pressure was noticed in Group B dogs. This could be due to relationship between the cardiomyopathies and blood pressure, where hypertension was noticed in cases with cardiomyopathy as per the findings of Sanchez *et al.* (2015). Similar findings were also noticed by Kuruvilla and Frankel (2003); Montoya *et al.* (2006). German *et al.* (2010) reported high blood pressure in obese cardiac diseased dogs.

The adopted procedure resulted in non-significant variation in the blood pressure within the groups *i.e.,* before and after VHS evaluation.

5.2.4 Hematological parameters

The mean values of all heamatological parameters varied non significantly between the groups. DeMorais, (2000), reported that hematology was not a good diagnostic tool for cardiac disease diagnosis, but it was useful to detect concurrent diseases and to guide the therapy. Similar opinions were recorded by Sisson *et al.* (2000), Ristic (2004). Deepti 2005), Boswood and Murphy (2006), Kumar *et al.* (2013) and Rajamohan (2018).

Non-significant variations in haematological parameters within the groups may indicate no evidence of acute somatic effect of radiation and stress leukogram during the procedure. This was in concurrence with the findings of Kuhn *et al.* (1991) and Beerda *et al.* (1997), who noticed variations in haematological parameters when the animals were subjected to stress.

5.2.4.1 Total Erythrocyte Count (TEC) (millions/cmm)

The mean values of TEC varied non-significantly between the groups. This was in concurrence with the observations of Boswood and Murphy (2006).

The adopted procedure resulted in non-significant variations in TEC within the groups *i.e.,* before and after VHS evaluation. But the slight increase in mean value TEC was observed after VHS evaluation in both groups, this is in concurrence with findings of Stockham and Scott (2008), who reported that physiological erythropoiesis was the state which resulted from normal physiologic responses to excitement, fright, and exercise that causes epinephrine release from adrenal medulla leading to increased erythrocyte number and concentration in the peripheral blood.

5.2.4.2 Haemoglobin (g/dL)

The changes in mean values of haemoglobin were comparable between the groups but varied non-significantly. However, Stafford *et al.* (2004) reported anaemia in dogs affected with idiopathic pericardial effusion and Sesh *et al.* (2013) reported decreased haematocrit values in dogs with DCM.

The adopted procedure resulted in non-significant variations in haemoglobin values within the groups *i.e.,* before and after VHS evaluation. As per Stockham and

Scott (2008) increased RBC levels in peripheral blood, physiological erythropoiesis may be found along with the physiological leucocytosis during stress/excitement/fear.

5.2.4.3 Packed cell volume (PCV) (%)

The mean values of PCV varied non significantly between the groups. This was in concurrence with findings of Boswood and Murphy (2006), who reported no significant alterations in PCV values in advanced stages of heart failure in 92 dogs.

The adopted procedure resulted in non-significant variations in PCV within the groups *i.e.,* before and after VHS evaluation. According to Stockham and Scott (2008), PCV raises significantly due to release of epinephrine due to stress, excitement and exercise, which leads to transient erythropoiesis in animals, but returned to normal levels after the stimulus is removed.

5.2.4.4 Total Leucocyte Count (TLC) (thousands/cmm)

There was comparable but non-significant changes between the groups which were within the normal reference range. This was in concurrence with findings of Ristic (2004) and contrary to findings of Glinska *et al.* (2006).

The adopted procedure resulted in non-significant variations in TLC within the groups *i.e.,* before and after VHS evaluation. As per Kuhn *et al.* (1991) and Beerda *et al*. (1997), specific changes in the number of peripheral neutrophils, lymphocytes and eosinophils may indicate acute stress in dogs.

5.2.4.5 Differential Leucocyte Count (DLC)

The variations were non-significant both within and between the groups.

5.2.4.5.1 Neutrophils (%)

Non-significant variations in neutrophils were noticed between the groups. This was in agreement with observations of Ristic (2004). However, Sisson and Kittleson (1999) reported the complete blood count of dogs with DCM was same as the normal dogs except for an occasionally a uncertain neutrophilia.

The adopted procedure resulted in non-significant variations in neutrophils within the groups *i.e.,* before and after VHS evaluation. According to Stockham and Scott (2008) stress neutrophilia resulted due to fear, excitement, pain, anxiety, fright and exercise.

5.2.4.5.2 Lymphocytes (%)

Non-significant variations in lymphocytes were noticed between the groups. This was in concurrence with Rustic (2004). However, Sisson and Kittleson (1999) reported that complete blood count of dogs with DCM was same as the normal dogs except for a moderate lymphopenia.

The adopted procedure resulted in non-significant variations in lymphocytes within the groups *i.e.,* before and after VHS evaluation. As per Stockham and Scott (2008), stress lymphopenia was caused by the change in lymphocyte kinetics caused by endogenous glucocorticoids.

5.2.4.5.3 Monocytes (%)

Non-significant variation in monocytes were observed between the groups**.** The adopted procedure resulted in non-significant variation in monocytes within groups *i.e.*, before and after VHS evaluation. As per Stockham and Scott (2008) stress monocytosis was caused by physiologic or neurogenic stress in dogs due to release of glucocorticoids.

5.2.4.5.4 Eosinophils (%)

Non-significant variation in eosinophils were observed between the groups. The adopted procedure resulted in non-significant variation in eosinophils within groups, before and after VHS evaluation. As per Stockham and Scott (2008), eosinopaenia might be part of either acute inflammatory or stress or steroid leucogram.

5.2.4.5.5 Basophils (%)

Non-significant variation in basophils were observed between the groups. The adopted procedure resulted in non-significant variation in basophils within groups, before and after VHS evaluation.

5.2.5 Biochemical tests

The adopted procedure had least effect on hepatic or renal system which resulted in non-significant variations in all biochemical parameters within the groups before and after VHS evaluation.

There was comparable but non-significant variations in values of biochemical parameters between the groups. This was in concurrence with DeMorais (2000), who opined that the routine biochemical parameters may remain normal in cardiac diseases except for slight elevation in liver enzyme and creatinine. However, they can be helpful to investigate potential concurrent disease as reported by Dukes-McEwan *et al.* (2003); Boswood and Murphy (2006) and Deepti (2015).

5.2.5.1 Serum creatinine (mg/dL)

The mean serum creatinine values varied non significantly between the groups. However Boswood and Murphy (2006) reported that serum creatinine concentrations changes significantly with advancement of heart failure and those changes reflects the transition from heart disease to heart failure.

Spangenberg *et al.* (2006) recorded no effect of activity and behaviour on serum creatinine. Creatinine was unchanged or little affected by a single dose of non-steroidal anti-inflammatory drugs (Lobetti and Joubert, 2000) and moderately increased by furosemide administration (Adin *et al.,* 2003). Since no drugs were administered during the procedure. The adopted procedure resulted in non-significant variations in serum creatinine values within the groups *i.e.,* before and after VHS evaluation.

5.2.5.2 Blood Urea Nitrogen (mg/dL)

The mean values of BUN were comparable and varied non-significantly between the groups. This was in agreement with findings of Dunn *et al.* (1999) and Sisson *et al.* (2000), who observed modestly elevated BUN in dogs with DCM.

BUN was unchanged by halothane anaesthesia in dogs (Loberti and Lambrechts, 2000). The VHS evaluation was carried on conscious dogs without anaesthetics and sedatives, and the adopted procedure resulted in non-significant variations in BUN within the groups *i.e.,* before and after VHS evaluation.

5.2.5.3 Alanine aminotransferase (ALT) (U/L)

The mean values of ALT varied non-significantly between the groups. This could be due to ALT is not a specific indicator of myocytes damage, and gives information about the extent of liver damage. This was in concurrence with findings of Stockham and Scott (2008). However Tidholm and Jonsson (1997) reported that an increased ALT can also be due to injury to cardiac and skeletal muscle as well. Gaskill *et al.* (2005) reported an increased ALT activity in dogs treated with phenobarbital.

The adopted procedure *i.e.,* the VHS evaluation was carried out in conscious dogs without use of anaesthetics or sedatives resulted in non-significant variations in ALT within the groups *i.e.,* before and after VHS evaluation. This could be due to least influence of procedure on hepatic system.

5.2.6 Diagnostic parameters

5.2.6.1 Thoracic radiography

In Group A, the cardiac silhouette appeared to be oval or egg shape in 83.33% of dogs (5 out of 6) in right lateral recumbency whereas in left lateral recumbency, it became more circular in shape in 66.67% of dogs (4 out of 6). This was in concurrence with Berry *et al.* (2009), who reported more circular heart LL view could be due to tendency of displacement of apex of cardiac silhouette from sternum in LL recumbency.

Heart of normal Labrador retriever breed of dogs occupied 3 intercostal spaces in 66.67% of the cases and 3.5 intercostal space in 33.33% cases in both lateral thoracic views. This is in line with findings of Owens (1985) and Kealy (1987), who used 2.5 to 3.5 intercostal spaces technique for dogs with a deep and wide thorax respectively as an indicator of normal heart size in lateral radiographic views.

In Group B, dogs with right ventricular enlargement (3 cases) showed increased convexity and increased sternal contact of the cranial cardiac margin in both lateral radiographic projections. This was in agreement with findings of Dunn *et al.* (1999) and Lord and Suter (1999). There was a cardiac bulging at 6 O’clock to 9 O’clock position in right ventricular enlargement in lateral thoracic radiographs. This was in agreement with findings of Poteet (2007). The right ventricular enlargement contributed to mild generalized radiographic cardiomegaly. Similar findings were also reported by Carlsson *et al.* (2009).

In dog with left atrio-ventricular enlargement (1 case) showed loss of caudal waist, cardiac bulging from 1 O’ clock to 6 O’clock position, trachea appeared parallel to thoracic spine and caudal cardiac margin straighter and more vertical. This was in agreement with findings of Hansson (2004) and Hansson *et al.* (2009).

In dog with biventricular enlargement (1 case) showed rounding of cardiac silhouette and increased sternal contact. This was in agreement with Bomassi (2007).

In dog with right atrial enlargement (1 case) showed cardiac bulging from 10 O' clock to 11 O'clock position. This was in agreement with findings of Berry *et al.* (2009) and Hansson *et al.* (2009).

5.2.6.1.1 VHS in Group A dogs

The right lateral and left lateral VHS of Group A was 10.1 ± 0.03v and 9.8 ± 0.03v which was higher than the generalized average value (9.7± 0.5v) of all dog breeds stated by Buchanan and Bucheler (1995). These findings were in line with Jaspen-Grant *et al.* (2013); Neagu *et al.* (2015); Begum and Bhuvaneshwari (2018) and Mourya *et al.* (2018). Similar results in Labrador retriever breed of dogs were reported by Gugjoo *et al.* (2013a) as 10.39 ± 0.05v and 10.29 ± 0.04v in right and left lateral VHS respectively. Further they recorded the mean ± SE values for short axis as 4.66 ± 0.02v and 4.67 ± 0.04v and long axis as 5.65 ± 0.03v and 5.75 ± 0.03v on both left and right lateral recumbency respectively. Nabi *et al.* (2014) reported the VHS values for some breeds of India viz., Labrador retriever as 10.21 ± 0.10v (RL), 9.93 ± 0.14v (LL). Neagu *et al.* (2015) reported 10.1v in right recumbency. Bodh *et al.* (2016) reported VHS in LL and RL radiographs as 10.22 ± 0.20v and 10.39 ± 0.19v respectively. Begum and Bhuvaneshwari (2018) reported 10.16 ± 0.36v in right lateral recumbency.

5.2.6.1.2 VHS in Group B dogs

The VHS in dogs with cardiac disorders was significantly greater than the control group. The average VHS of Group B was 11.8 ± 0.17v and 11.3 ± 0.18v in right lateral and left lateral recumbency respectively. The elevated VHS in Group B dogs is due to the cardiomegaly contributed by dilatation of heart chambers in cardiac disease affected dogs. Similar findings were reported by Bright and Mears (1997), who noticed that dogs with dilated cardiomyopathy typically had generalized cardiomegaly radiographs. Bodh *et al.* (2014) reported increased VHS of 11.55 ± 0.28v and 11.24 ± 0.25v in right and left lateral recumbency in Labrador retriever dogs with occult DCM.

The elevated VHS in different cardiac affections in other breeds were reported by Guglielmini *et al.* (2009) reported VHS >11.4v in chronic mitral valve disease insufficiency, Tai and Hwang (2013) reported 12.7 ± 1.69v in CDMD dogs, Carlsson *et al*. (2009) in small breed of dogs with mitral valve disease with VHS 13.2 ± 1.1v, Guglielmini *et al.* (2012) reported >11.9v in mild pericardial effusion, Cote *et al.* (2013) reported VHS >10.7v in dogs with cardiac tamponade attributed to pericardial effusion. Torad and Hassan 2014) reported 10.5 ± 0.4v (10.2+10.9v) in German shepherd breed affected with cardiomegaly, Bodh *et al.* (2016) recorded the right lateral VHS as 13.10v and left lateral VHS as 12.80v in Doberman pinscher dogs with generalized cardiomegaly and congestive heart failure. Ingole *et al.* (2016) reported 12.12 ± 0.17v in DCM affected dogs.

5.2.6.1.3 Effect of right lateral and left lateral recumbency on VHS

The right lateral VHS was significantly greater than left lateral VHS in both the groups. This could be explained by the fact that divergence of X-ray beam and more distance of the heart from the cassette occurs in right lateral recumbency, which leads image magnification. This was in concurrence with Gulanber *et al*. (2005) and Gugjoo *et al.* (2013a).

Suter and Lord (1984) and Kraetschmer *et al.* (2008) opined that gravity and tilting of the heart within the thorax due to different recumbent positions also influence the cardiac silhouette. Slight but noticeable differences have been described between radiographs made in right versus left lateral recumbency.

The greater VHS in right lateral recumbency than left lateral recumbency was contributed by significant increase in the long axis of the right lateral recumbency than that of left lateral recumbency. This was in agreement with findings of Spencer *et al.* (1981), who reported that differences between the appearances of thoracic radiographs made in right and left lateral recumbency may be result of compression of lung by overlying or adjacent structures due to the force of gravity. The cardiac silhouette did not change consistently in size or shape, however, the heart usually appeared longer from apex to base in right lateral recumbency due to the placement of cardiac apex slightly away from the midline, which offers a slightly smaller profile to the X-rays in left lateral recumbency. Due to this, it was reasonable to expect that the cardiac apex would be closer to the sternum in right lateral recumbency. This was in concurrence with Berry *et al.* (2009). Because of this finding, right lateral recumbency may provide the most accurate information for cardiac size evaluation.

The short axis *i.e.*, cardiac width in right and left lateral recumbency varied non significantly within the groups and the cardiac width remained almost same in right and left lateral recumbency in both the groups.

5.2.6.2 Electrocardiography

Electrocardiographic parameters of Group A were found to be within the normal reference range for Labrador retriever breed of dogs. This was in line with findings of Gugjoo *et al.* (2014a) who provided normal reference range of electrocardiographic parameters in Labrador retriever breed of dogs. The mean values of ECG parameters varied non-significantly between the group but comparable changes were noticed in group B dogs. Similar findings were also noticed by Mukherjee *et al.* (2020) in Labrador retriever breed of dogs.

P mitrale (wide P wave >0.04 seconds in duration) was noticed in one dog (B5) with DCM which had evidence of left atrio-ventricular enlargement. This was in agreement with findings of Tilley and Smith (2001) and Dukes-McEwan (2000), who reported that P mitrale could be seen chronic mitral valvular insufficiency and DCM. However Rajkumar (2013) reported that P wave electrocardiogram was not a sensitive indicator of left atrial enlargement.

Low voltage QRS complexes occurred in 3 dogs (B2, B3 and B5). They were characterized by short R waves which were < 0.5mV in amplitude. This was in concurrence with the findings of Tilley and Smith (2001), who opined low amplitude R waves may occur when transmission of the cardiac electrical impulse to the skin was hindered, this may occur with pleural effusion, pericardial effusion, pulmonary oedema, obesity or subcutaneous oedema.

Q dip (waves of > 0.5 mV amplitude) was noticed in one dog (B6) having right ventricular enlargement. This was in line with findings of Tilley and Smith (2001) and

Kumar *et al.* (2010), who reported that Q dips in electrocardiograms were sensitive indicator of right ventricular enlargement.

S dip or increased S amplitude > 0.35 mV indicative of right ventricular enlargement noticed in one dog (B2). This was in agreement with findings of Grady *et al.* (1992), Martin (2000) and Tilley and Smith (2001).

Decreased QRS duration was seen in one dog (B4) which had left ventricular enlargement. The same dogs showed left ventricular enlargement in thoracic radiography and in echocardiography. Similar findings were reported by Grady *et al*. (1992).

Arrhythmia was noticed in one case (B1), which had biventricular enlargement. This was in line with the findings of Rasmussen *et al.* (2012), who reported sinus arrhythmia was often present during progression to CHF in dogs.

Increased QT interval was noticed in one dog (B2), which had left ventricular enlargement. This was in agreement with Bodh *et al.* (2014), who reported (0.25 seconds) increased QT interval in Labrador retriever dogs with occult DCM and opined that this could be due to higher mean heart rate (102.18 ± 4.94 beats per minute) in these dogs.

5.2.6.3 Echocardiography

In our study echocardiography was used for subjective assessment of cardiac function and anatomy and as a supporting diagnostic tool. In Group A dogs, the 2D and M-mode echocardiographic parameters were within normal reference range for Labrador retriever breed of dogs. This was in concurrence with the findings of Gugjoo *et al.* (2014b).

In Group A dogs, the 2D echocardiography revealed straight inter ventricular septum which indicate no evidence of ventricular dilatation and volume or pressure overload. The left ventricular wall and inter-ventricular septum were similar in size which suggests no evidence of dilatation or thinning of left ventricle. Right ventricular chamber was one third to one half the size of the left ventricular chamber at their largest dimensions suggesting normal right ventricular volume and no evidence of distension. The similar sized aorta and left atrium indicated no evidence of distension of left atrium. Mitral valve leaflets were thin and had same thickness from their base to their tips during diastole, which suggested no evidence of valve defect. The right parasternal short axis view revealed symmetrical and circular outer shape of the left ventricle, curved ventricular septum, mushroom shaped left ventricle which suggested no evidence of ventricular dilatation. The left parasternal long axis view revealed the right auricles cleared of soft tissue densities which suggests no evidence of mass in right atrium. The M mode measurements of left ventricle were within the prescribed standard value for the respective body weight. The contractility of the ventricles was normal. The above echocardiographic findings were in agreement with observations of Gugjoo *et al.* (2014b) and Boon (2017).

The significant increase in LVIDd, LVIDs, EDV, ESV and LA/Ao ratio whereas significant decrease in EF (%) and FS (%) was noticed between the groups. These were indicative of reduction of ventricular function/contractility and its enlargement, increase in LA/Ao ratio (1.56 ± 0.08) was indicative of left atrial enlargement. These were in concurrence with the findings of Bodh *et al.* (2014). Similar findings were reported by

Borgarelli *et al.* (2008); Rajkumar (2013); Cubas *et al.* (2017); Chetboul *et al.* (2018) and Keene *et al.* (2019).

The right parasternal long axis view of Group B dogs with right ventricular enlargement showed downward curvature of inter ventricular septum suggestive of right ventricular volume overload in three cases, the aorta and left atrium were similar in size suggestive of no evidence of left ventricle distension, the right ventricular chamber was almost of equal size of left ventricle which indicates the dilatation of right ventricle. The right parasternal short axis transverse view revealed flattened inter-ventricular septum which suggests the depression of inter-ventricular septum by right ventricular dilatation. This was in concurrence with the findings of Pyle *et al.* (2004).

In dogs with left atrio-ventricular dilatation (1 dog), there was a increased left ventricular internal dimension, elevated inter-ventricular septum which could be due to lifting of inter-ventricular septum due to left ventricle distension. In dogs with biventricular dilatation (1 dog), the M mode echocardiography revealed marked dilatation of both ventricles suggestive of biventricular dilatation. One dog showed thickened mitral valve and regurgitation of blood at mitral valve with left atrial enlargement indicative of myxomatous mitral valve disease (MMVD). These observations were in accordance with observations of Calvert *et al.* (1982); Chetboul *et al.* (2004); Pyle *et al.* (2004); Fine (2008) and Rajkumar (2013) in DCM and MVD affected dogs.

5.2.7 Correlation of VHS findings with ECG and echocardiography

For objective correlation of VHS with ECG and echocardiography, it was necessary to have the same type of cardiac disorders in all Labrador retriever breed of dogs in Group B. During the period of study, the cardiac diseases recorded in Labrador retrievers were ventricular enlargement involving right ventricle (3), left atrium and ventricle (1), both ventricles (1) and right atrial enlargement (1) which were not sufficient for proving the existing correlation between VHS, ECG and echocardiography by statistical method. Hence further studies were needed to prove the objective correlation existing between the above parameters.

In the present study, it was evident that VHS was subjectively correlated well with electrocardiography and echocardiography findings in both the groups which was concluded by the following observations.

In Group A, Labrador retriever breed of dogs with normal cardiac silhouette in thoracic radiography. ECG parameters varying within the reference range of Labrador retriever breed of dogs and the normal cardiac function and internal cardiac structures with normal reference values of echocardiography for Labrador retriever dogs showed a VHS of 10.1 ± 0.03v and 9.8 ± 0.03v in right and left lateral recumbency respectively.

In Group B, Labrador retriever breed of dogs suffering from dilated cardiomyopathy with radiographic cardiomegaly, altered ECG parameters and showed dilatation of ventricles, altered parameters and functions of chambers of heart (ventricles) in all cases and MMVD in one case showed significantly increased VHS values *i.e.*, 11.8 ± 0.17v and 11.3 ± 0.18v in right and left lateral recumbency respectively.

Nakayama *et al.* (2001) designed a study to determine how well echocardiographic and electrocardiographic findings correlated with VHS and noticed a strong correlation of VHS with P wave duration and QRS duration of ECG and left atrium-to-aorta ratio, left ventricular end-diastolic diameter, left ventricular end-systolic diameter of echocardiography in 16 dogs with induced cardiomegaly by rapid ventricular pacing. They further concluded that change in any parameter obtained from thoracic radiographs, ECGs, or ECHOs possibly could be due to changing orientation of the heart within the thorax as cardiomegaly increases. These values influenced by position of heart within the thorax, which changes the projection of heart on the radiographic planes and on lead axes of the ECG or the axis of the probe through the heart and thoracic wall. Saida *et al.* (2008) reported a significant positive relationship between VHS and echocardiographic parameters like LA/Ao, peak E wave velocity, peak A wave velocity cardiac index, left ventricular end diastolic volume index, stroke volume index and heart rate. Bavegems *et al.* (2009) reported a significant correlation between the R wave amplitude in lead II with the VHS from left lateral and right lateral thoracic radiographs and left ventricular internal diameter in systole and diastole from echocardiography

However, Buchanan and Bucheler (1995) concluded VHS was superior to echocardiography in objectively measuring the progression of heart disease. Birks *et al*. (2017) reported that non-significant correlation between left atrial echocardiographic parameters and VHS of Dachshund dogs.

Summary

VI. SUMMARY

- A total of 8472 dogs were presented to the Veterinary College Hospital, KVAFSU, Hebbal, Bengaluru during June 2017 to May 2018. Among these, 165 (1.94%) dogs were diagnosed to be affected with cardiac diseases

- Breed-wise, Labrador retriever breed of dogs represented the highest 24.84% of cardiac diseased dogs. The middle aged (4-8 years) dogs were overrepresented (43.63%) followed by old dogs (> 8 years) (35.15%) and the least occurrence was in young dogs (0-4 years) (21.21%). Male Labrador retrievers had higher occurrence than female ones.

- The clinical, physiological and haemato-biochemical parameters varied non significantly both within and between the groups except body weight, heart rate and systolic blood pressure. These parameters varied significantly between the groups and non-significantly within the group *i.e.*, before and after VHS evaluation.

- Thoracic radiography revealed normal cardiac silhouette in Group A dogs. But right ventricular enlargement (3 dogs), biventricular enlargement (1 dog), left atrio-ventricular enlargement (1 dog) and right atrial enlargement (1 dog) in Group B dogs.

- VHS in healthy normal Labrador retriever dogs (Group A) was 10.1 ± 0.03v and 9.8 ± 0.03v in right and left lateral recumbency respectively. VHS in Labrador retriever dogs with established cardiac disorders (Group B) was 11.8 ± 0.17v and 11.3 ± 0.18v in right and left lateral recumbency respectively. The VHS of Group B dogs was significantly greater than the Group A dogs.

- The VHS in right lateral recumbency was significantly greater than left lateral recumbency. This could be due to significantly elevated long axis of heart in right lateral recumbency as compared to left lateral recumbency. The short axis varied non significantly both within and between the groups.

- The ECG parameters varied non significantly between the groups but comparable alterations were noticed. The echocardiography showed significant increase in LVIDd, LVIDs, EDV, ESV and LA/Ao ratio and significant decrease in EF (%) and FS (%) was noticed between the groups.

- The VHS was subjectively correlated with the ECG and echocardiographic parameters for Labrador retriever breed of dogs.

In conclusion, VHS is easily applicable, interpretable, non-invasive and accurate method for diagnosing cardiac disorders in thoracic radiographs. Right lateral recumbency provided the most accurate information for cardiac size evaluation as there was displacement of cardiac apex from sternum in left lateral recumbency. Hence, the right lateral VHS can be considered as standard view for interpretation of cardiac diseases by thoracic radiography.

The VHS provided the radiographic cardiac silhouette incorporating all the chambers, including myocardium and pericardium. The electrocardiography provided electrical activities of heart and echocardiography provided the myocardial function and intracardiac dimensions. Therefore combination of all three techniques will help in assessing the severity of heart disease and aid in evaluation of therapeutic efficiency.

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Abstract

VIII. ABSTRACT

The study was carried out in Labrador retriever breed of dogs to study the influence of right and left lateral recumbency on Vertebral Heart Score. Twelve Labrador retriever dogs of either gender were selected for the study and divided into two groups, A and B consisting of six dogs each. Group A included six normal Labrador retriever dogs and Group B included six Labrador retriever dogs with established cardiac disorders. The overall occurrence of cardiac disorders among the dogs was 1.94% with Labrador retrievers represented the highest occurrence of cardiac diseased dogs (24.84%) in one year study period. The middle aged (4-8 years) dogs were overrepresented (43.63%) followed by old dogs (> 8 years) (35.15%) and the least occurrence was in young dogs (0-4 years) (21.21%). Male Labrador retrievers had higher occurrence than female ones. VHS in Group A dogs was 10.1 ± 0.03v and 9.8 ± 0.03v in right and left lateral recumbency respectively. VHS in Group B dogs was 11.8 ± 0.17v and 11.3 ± 0.18v in right and left lateral recumbency respectively. The VHS of Group B dogs was significantly greater than the Group A dogs. The VHS in right lateral recumbency was significantly greater than left lateral recumbency. The ECG parameters varied non significantly between the groups but comparable alterations were noticed. The echocardiography showed significantly increased LVIDd, LVIDs, EDV, ESV and LA/Ao ratio and significantly decreased EF (%) and FS (%) between the groups. The VHS was subjectively correlated well with the ECG and echocardiographic parameters for Labrador retriever breed of dogs. Finally, based on observations and evaluations, it could be concluded that the combination of all three techniques was helpful in assessing the severity and significance of heart diseases and useful to evaluate the therapeutic efficiency.