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RESEARCH ARTICLE

DIAGNOSTIC EVALUATION OF DILATED CARDIOMYOPATHY IN LABRADOR RETRIEVERS

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Abstract

The objective of the present study is to record the incidence, clinical presentation, electrocardiographic, radiographic, laboratory, two dimensional echocardiography, M-mode echocardiography, pulsed wave doppler and color flow doppler in Labrador retrievers with Dilated Cardiomyopathy. It included 30 healthy dogs and 53 confirmed cases of DCM out of which 30 cases were randomly selected for the study. Prevalence of dilated cardiomyopathy in Labrador Retrievers was found to be 6 per cent in the present study. On radiography, cardiomegaly and pulmonary edema were the major findings observed. In echocardiography, increased left ventricular end diastolic dimension and systolic dimension, reduced fraction shortening, increased E-point sepal separation, increased LA/AO ratio, decreased ejection fraction, increased end diastolic volume and end systolic volume were noticed. On pulsed wave doppler echocardiography reduced PA, AO, LVOT, RVOT velocities were recorded. Mild to moderate regurgitation was observed in Mitral and Tricuspid valve by color flow doppler echocardiography. From the present study it was concluded that radiography acts as an important aid in diagnosing pulmonary edema and in echocardiography two dimensional derived volumes and ejection fraction were found to be more reliable in Dilated Cardiomyopathy of Labrador retrievers. M-mode derived chamber dimensions, E-point septal separation and Fractional shortening remains as a gold standard for diagnosing Dilated Cardiomyopathy in Labrador Retrievers. Pulsed wave doppler and color flow doppler were useful in assessing velocity and flow pattern across valves

INTRODUCTION

Idiopathic, Dilated Cardiomyopathy (DCM) is a condition of unknown etiology characterized by a progressive dilatation of one or both ventricles with severe impairment of systolic function in the absence of congenital, coronary arterial, hypertensive, vascular, pulmonary parenchymal, valvular, or other cardiovascular disorders(Cobb, 1992). Canine DCM has long suspected to have a genetic basis and various modes of inheritance

have been reported. Autosomal dominant mode of transmission has been reported in Newfoundland (McEwan, 1998). Anecdotal reports suggest that incidence of DCM in Labrador retrievers were more common than other breeds at Madras Veterinary college teaching hospital and same mode of inheritance may be suspected as it has originated from Newfoundland. Till date no published reports were available on Dilated Cardiomyopathy of Labrador retrievers. The ability to diagnose DCM is increasing with the increasing availability of ultrasound in general practise. The clinical signs and presentations are variable and usually the prognosis is grave. In practise, diagnosis is made on the basis of clinical findings and echocardiographic examinations as opposed to histopathology following post-mortem which is now rarely permitted by owners. Presently Dilated Cardiomyopathy is reported to affect young to middle aged male dogs and clinical signs often occur acutely which includes dyspnoea, coughing, syncope, exercise intolerance and abdominal distension. The prevailing radiographic picture consists of generalised cardiomegaly and pulmonary edema. Atrial fibrillation remains as the most common electrocardiographic abnormality. Echocardiographic findings consist of markedly depressed left ventricular contractility and dilation of cardiac chambers (Keene, 1989). The present study may help to build a basic data on Dilated Cardiomyopathy of Labrador retrievers, So that in future these data may help in doing research on etiology and therapy. Moreover this study may create awareness among practising veterinarians and henceforth the quality of practise may improve.

Materials and Methods

Study was carried out at Madras Veterinary College Teaching Hospital, Tamil Nadu Veterinary Animal Sciences University (TANUVAS) comprised of healthy and DCM Labrador retriever dogs. In one year period the number of Labrador retriever attended Madras Veterinary College Teaching Hospital was 873, out of which 53 dogs were diagnosed with dilated cardiomyopathy. Among 53 dogs, 30 dogs were randomly allotted to the study group. For control group, 30 apparently healthy male and female Labrador retrievers aged between 4 and 8 years presented to the hospital for health check-up and vaccination were taken for the assessment of baseline referral values of selected parameters. The study consisted of two groups which included apparently healthy Labrador Retrievers (Group I) and clinical cases of Dilated Cardiomyopathy (Group II). All the animals were subjected to clinical examination, laboratory investigation, radiography, electrocardiography, doppler blood pressure and echocardiography. The postmortem examination was carried out in dogs which died during the study period. Laboratory investigation (haematology and biochemical) were conducted as per standard protocol using auto analyzer. The animals under study were subjected to thoracic radiography to record the changes and Vertebral Heart Score (Figure-1) was calculated (Buchanan and Bucheler, 1995). Electrocardiography was recorded as per the standard procedure (Tilley and Smith, 1995).

Systolic blood pressure measurement by Doppler method

The Instrument Used for the present study was Vet- Doppler ultrasound blood pressure machine manufactured by company V med technology. Animal was placed on right lateral recumbency, either forelimb or hind limb was used. The cuff whose width was about 40 percent of the limb circumference was used for blood pressure measurement. As the superficial palmar arterial arch is used for blood pressure measurement, the hair over the area distal to palmar meta carpel pad was clipped and the appropriate sized cuff was positioned over the mid-ante brachium. Ultrasound gel was applied to get good contact between the skin and the transducer. The cuff is attached to a pressure manometer and inflated and deflated manually. The first sound heard as the cuff gradually deflates is recorded as the systolic pressure. The diastolic pressure is estimated by a change in the character of the pulsing sound as the cuff deflates.

Echocardiography

Echocardiographic examinations were performed as suggested by (Boon, 1998) using ALOKA SSD 3500 ultra sound system with a cardiac probe of 3.0 – 6.0 MHz to obtain Two dimensional, M- mode, Pulsed wave and color flow Doppler echocardiography images of heart.

Preparation and positioning of the animal

Immediately after arrival to the hospital, the patient was taken to a calm and isolated area to minimize the stress of transport. An area of hair coat was clipped between the costochondral junction and the sternum on both sides of the thorax in the area of the right and left parasternal windows and all the echocardiographic measurements were carried out with the animal lying in lateral recumbency. Each animal was positioned in right and left lateral recumbency, and imaged from below through a notch in the table (Koch *et al.*,1996).

Two dimensional echocardiography

It was done at right parasternal long axis view which was obtained at the fourth and fifth intercostal spaces ventrally between the sternum and the costochondral junction. From the long-axis views, the index marker of the transducer was directed towards the elbow of the patient to obtain short-axis views. (Figure-2). Two dimensional measurements for AO and LA were obtained from a short-axis plane at the level of the aortic valves. The first frame

after the aortic excursion was used, which is early diastole. In 2-D, transverse dimensions of AO and LA were measured. For AO, the first caliper was placed at the midpoint of the convex curvature of the wall of the right aortic sinus. The second caliper was positioned at the point where the aortic wall and the non-coronary and the left coronary aortic cusps merge. The LA was measured by extending the line from the same point where the second caliper was positioned to the blood-tissue interface of the LA wall. The LA/AO ratios were calculated as an index for atrial size (Koch *et al.*, 1996).

Simpson's method of Disc (SMOD)

Left apical four chamber view was used. It was obtained by placing the transducer over the apex, the ultrasound beam was directed almost perpendicular to the sternum and parallel to the long axis of the heart with reference mark directed toward the spine and the crystals pointing towards the neck (cable to knees), about 30 degrees between chest wall and transducer (Figure-3). By rotating the ultrasound beam in a left caudal to right cranial direction and angling dorsally towards the base of the heart, a four-chamber view was obtained showing the left ventricle and atrium on the right side and the right ventricle and atrium on the left side (Koch *et al.*, 1996). SMOD measurements were done on left apical four chamber view with selection of end diastolic frames (at the time of mitral valve closure) and end-systolic frames (corresponding to the last frame before mitral valve opening). The LV area was measured by tracing the endocardial border on each selected image; maximal LV length was measured from the middle of a line connecting the two mitral annuli to the endocardial border of the LV apex. LV volumes were then automatically calculated by the ultrasound machine. Ejection Fraction was calculated from SMOD derived end-diastolic and end-systolic LV volumes by using the following formula:

$$\text{Ejection Fraction} = \frac{\text{EDV} - \text{ESV}}{\text{EDV}} \times 100\ \% \quad \text{(Boon 1998)}$$

M-mode echocardiography

From the long-axis view, the transducer was rotated 90° in clockwise direction and the ultrasound beam was orientated perpendicular to the long axis of the ventricles. Proper alignment was achieved if the left ventricle and the aortic root are displayed as round structures (Figure-4).The examination normally begins at the level of the apex of the heart, followed by planes through the papillary muscles, chordae tendineae, mitral valve, and main pulmonary artery. For these views, the transducer was angled progressively from ventral to dorsal; towards the heart base. The view through the papillary muscles is described as having a mushroom shape, while the view through the mitral valve is known as the fish mouth view (Koch *et al.*, 1996).

M-mode images were obtained from the real time left ventricular transverse plane by placing the cursor over the structures. The resulting M-mode imaging as depth through the heart on the Y- axis and time on the X – axis and the following measurements were collected: E-point septal separation, Left ventricular diameter at end diastole (LVIDd), left ventricular diameter at end-systole (LVIDs), left ventricular posterior wall thickness at end-diastole (LVPWd), left ventricular posterior wall thickness at end-systole (LVPWs), interventricular septal thickness at end-diastole (IVSd) and interventricular septal thickness at end-systole (IVSs). From these above values the contraction indices like fraction shortening, interventricular septum fraction thickening and left ventricular posterior wall fraction thickening were calculated using the following formulas (Koch *et al.*, 1996).

$$\text{Fractional shortening} = \frac{\text{LVIDd} - \text{LVIDs}}{\text{LVIDd}} \times 100\ \%$$

$$\text{Interventricular septum fraction thickening} = \frac{\text{IVSs} - \text{IVSd}}{\text{IVSd}} \times 100\ \%$$

$$\text{Left ventricular posterior wall fraction thickening} = \frac{\text{LVPWs} - \text{LVPWd}}{\text{LVPWd}} \times 100\ \%$$

(Boon 1998)

Mitral Valve flow

Imaging plane: Left caudal (apical) four chamber view (Figure- 5). Sample volume position: In the left ventricle just distal to the mitral annulus at the point of maximal opening of the mitral valve.

Left Atrial Flow

Imaging plane: Left caudal (apical) four chamber view (Figure-6). Sample volume position: One quarter of distance between the mitral annulus and the dorsal wall of the left atrium.

Tricuspid Valve Flow

Imaging plane: Left caudal (apical) four chamber view (Figure– 7) . Sample volume position: In the right ventricle just distal to the tricuspid annulus at the point of maximal opening of tricuspid valve.

Right Atrial Flow

Imaging plane: Left caudal (apical) four chamber view (Figure-8). Sample volume position: One quarter of the distance between the tricuspid annulus and the dorsal wall of right atrium.

Aortic Valve Flow

Imaging plane: Left caudal (apical) long axis view of left ventricular outflow region. Occasionally an apical five chamber view was used to align more parallel to the ascending aorta (Figure-9). Sample volume position: In the ascending aorta at the distal end of the sinus of valsalva.

Left Ventricular Outflow Tract Flow

Imaging plane: Left caudal (apical) long axis view of left ventricular outflow region (Figure-10). Occasionally an apical five chamber view was used to align more parallel to the outflow tract and aorta. Sample volume position: In the left ventricle just proximal to the aortic valve and midway between the septum and open anterior mitral valve leaflet.

Pulmonary Valve Flow

Imaging plane: Right parasternal short axis view of the right ventricular outflow tract at the aortic valve level (figure-11). Sample volume position: In the pulmonary artery just distal to the valve.

Right Ventricular Outflow Tract

Imaging plane: Right parasternal short axis view of the right ventricular outflow tract at the aortic valve level (Figure-12). Sample volume position: In the right ventricle just proximal to the pulmonary valve and midway between the aortic wall and right ventricular wall.

Color flow Doppler echocardiography

It was performed in right parasternal long axis view, short axis view and left apical four chamber view by superimposing the colour flow Doppler beam over the real time-two dimensional images. Normal and abnormal flow pattern were assessed (Figure-13).

Statistical analysis

The data obtained were subjected to statistical analysis as per Snedecor and Cochran (1994). The results were presented in figures, tables and graphs.

Results and Discussion

Incidence

During the study period (August 2011-July 2012) the total no of Labrador retrievers were brought to Madras Veterinary college teaching hospital for various reasons was 873. In this 53 labrador retrievers were diagnosed to have dilated cardiomyopathy accounting for prevalence of 6 per cent. The average age of affected dogs was 6.68 ± 0.47years, and the incidence in male dogs were 68 per cent (36/53) and female dogs were 32 per cent (17/53).

In the present study the prevalence of DCM in Labrador retrievers was found to be 6 per cent with male predominance. The average age of the affected dog was 6.68 ± 0.47 years. Grady and Sullivan (2004) reported 0.3 per cent incidence of DCM in Labrador retrievers from the University of Purdue Veterinary Medical Database 1985 to 1991. The present finding is not in agreement with Grady and Sullivan. This higher prevalence of DCM in Labrador retrievers in this study may be due to the mode of inheritance in the ancestral population. McEwan, (2000) opined that in the most of the familial DCM, autosomal dominant mode of inheritance was suspected, whereas Ortiz-Lopez *et al.*,(1996) considered autosomal dominant, autosomal recessive, X-linked and mitochondrial modes of inheritance in the DCM affected dogs. Therefore the ancestral population would have been heterozygotes or homozygotes for DCM related inheritance. Even very high incidence of around 24 per cent was observed by Vollmar (200) in Irish wolfhounds. This gives a logical conclusion that the prevalence rate depends on the population under study.

In the present study the average age of DCM affected dogs was 6.68 ± 0.49 years. This concurs with the findings of Tidholm *et al*., (1997) where they observed DCM at a mean age of 6.6 years. Tidholm and Jonsson (1996) in a study of Newfoundland dogs with DCM reported the mean age of affected dogs as 5 years which is less than the average of dogs affected with DCM in the present study. The incidence of DCM in male dogs was higher compared to females in the present study. This is in agreement with Tidholm and Jonsson (1996) who studied DCM in 37 Newfoundland dogs and reported 62 per cent in males and 38 per cent in females.

History and clinical signs

Among 30 randomly selected cases of DCM the major history and clinical signs were abdominal distension in 80 per cent (24/30) of dogs, exercise intolerance in 70 per cent (21/30) of dogs, weight loss in 70 per cent (21/30) of dogs, cough in 60 per cent (18/30) of dogs, episodic weakness in 20 per cent (6/30) of dogs and syncope in 16 per cent (5/30) of dogs. The above findings are in agreement with the study in 187 dogs with DCM by Tidholm *et al.,* (1997).

The physical examination findings recorded were dyspnoea in 100 per cent (30/30) of dogs, ascites in 97 per cent (29/30) of dogs, gallop rhythm in 87 per cent (26/30) of dogs, systolic murmur in 73 per cent (22/30) of dogs, limb oedema in 53 per cent (16/30) of dogs and weak femoral pulse in 50 per cent (15/30) of dogs. These findings concur with several authors (Martin *et al.*, 1991; Martin *et al.*, 2010). Dyspnoea may be due to pulmonary edema subsequent to left sided failure and ascites due to right sided failure. Systolic murmur and low pitched pro diastolic (S3) gallop sound was auscultated as an evidence of severe ventricular diastolic impairment (Sisson, 1995).

Laboratory findings

The Mean ± S.E values of haematology and biochemistry in control and DCM dogs are given in Table- 1. Highly significant increase in the mean ± S.E values of white blood cell count and absolute neutrophil count were noticed in DCM dogs compared to control. Significant decrease in the haemoglobin, packed cell volumes and red blood cell count were observed in DCM dogs compared to control. No significant changes were noticed in mean ± S.E values of platelet, absolute lymphocyte count and absolute monocyte count in DCM dogs compared to control.

Highly significant increase in the mean ± S.E values of Blood urea nitrogen and Creatinine were noticed in DCM dogs compared to control. Significant decrease in the total protein and significant increase in the potassium were noticed in DCM dogs compared to control. No significant changes were noticed in mean ± S.E values of alanine aminotransferase, serum alkaline phosphatase, albumin, sodium, calcium and phosphorus in DCM dogs compared to control. In the previous studies several authors McEwan (200), Martin *et al*. (2009) recorded elevated blood urea nitrogen and Creatinine, elevated liver enzymes and hypoprotenemia. But in the present study although significant change were observed in certain biochemical parameters they were within the normal ranges.

Significant increase in WBC count and absolute neutrophil count were observed in dogs with DCM, whereas a significant decrease in Hb, PCV and RBC were observed. Leucocytosis and Neutrophilia recorded in the present study is in contrast to the findings of Martin *et al.*,(2010). These authors observed no haematological abnormalities in DCM affected dogs. The reason for this increase in the present study may be attributed to concurrent bacterial infection in few cases, which added to the increase in mean values for these parameters. Significant decreases in haematological values were observed in the present study. This concurs with the findings Martin *et al*.,(2009). These authors recorded low haematocrit values in less than 19 per cent of the dogs they studied.

Radiography

The Mean ± S.E values of vertebral heart score in control and DCM dogs are given in Table-2. Highly significant increase in the mean ± S.E values of vertebral heart score was noticed in DCM dogs compared to control. The other major radiographic abnormalities in the DCM affected dog were cardiomegaly in 100 per cent (30/30) of dogs, pulmonary edema in 80 per cent (24/30) of dogs and pleural effusion in 20 per cent (6/30) of dogs. These findings are in agreement with Tidholm and Jonsson (1996) and Martin *et al* .,(2010). Highly significant increase in the Vertebral Heart Score (12.5 ± 0.14) was observed in DCM dogs compared to normal (10.78 ± 0.03). Lamb *et al*., (2001) recorded Vertebral Heart Score of 10.6 in health Labrador retrievers which concurs with the present study. No reports are available on Vertebral Heart Score of Labrador retrievers in DCM. In the present study the Vertebral Heart Score was found to be reliable in diagnosing cardiomegaly. This finding is in contrast with Lamb *et al.*,(2000) who opined that the observer's accuracy of diagnosis did not change significantly as a result of using Vertebral Heart Score as an adjuvant to subjective assessment of radiography.

Electrocardiography

The Mean ± S.E values of electrocardiography in control and DCM dogs are given in Table-2. The Significant increase in the mean ± S.E values of Heart rate was noticed in DCM dogs compared to control. No significant difference in the mean ± S.E values of P- amplitude, P- duration, PR- interval, QRS- duration, R-amplitude, QT interval were noticed in DCM dogs compared to control. Normal sinus rhythm was appreciated in 63 per cent (19/30) of dogs and ST depression in 70 per cent of dogs that were affected with DCM. The abnormal rhythm which includes atrial fibrillation was present in 27 per cent (8/30) of dogs, ventricular premature contraction in 10 per cent (3/30) of dogs.

ST depression was consistently observed in 70 per cent of dogs with DCM as an indication of cardiomegaly. Major rhythm disturbances observed were AF in 27 per cent of dogs and VPC in 10 per cent of dogs. Majority of the dogs (63 per cent) showed normal sinus rhythm. The findings in the present study is in agreement with several authors which includes Sisson and Thomas (1995), Tidholm and Jonsson (1997) and Calvert and Wall (2001) . They all observed AF and VPC as the common arrhythmias in dilated cardiomyopathy affected dogs across the breeds. However, no much change in the other quantitative parameters to assess the enlargement pattern was seen and also 63 per cent of dogs had normal sinus rhythm. Therefore, it is concluded that ECG was not effective in diagnosing DCM whereas warranted in identifying arrhythmias. Severe left atrial dilatation in DCM dogs was the reason behind the increased incidence of AF in dogs with DCM. It has been suggested that susceptibility to AF increases with increased atrial mass (1984).

Echocardiography

The Mean ± S.E values of two dimensional, M mode and pulse wave doppler echocardiography values in control and DCM dogs are given in Table-3. Highly significant increase in the mean ± S.E values of LA (Figure-14), Ao, LA/Ao ratio (Figure- 15), EDV and ESV (Figure-16) were noticed in DCM dogs compared to control. Highly significant decrease in the mean ± S.E values of EF was noticed in DCM dogs compared to control. No significant difference in the mean ± S.E values of SV was noticed in DCM dogs compared to control.

Highly significant increase in the mean value of LA (4.09 ± 0.05cm) Ao (2.08±0.05cm), LA/Ao (2.01±0.06), EDV (95.16±1.97ml) and ESV (67.30±2.36ml) and highly significant decrease in the ejection fraction (29.11±2.13 per cent) were observed in DCM affected dogs compared to control. These findings are in partial agreement with Borgarelli *et al.* (2006) in a study of 63 dogs with DCM reported EF as 27.7 per cent. McEwan *et al.*, (2003) suggested that less than 40 per cent of EF determined by modified Simpson's rule was abnormally low. In the present study, DCM dogs showed an ejection fraction of 29.11 ± 2.13 per cent when compared to 52.81 ± 0.73 per cent in control dogs which is in agreement with the above study.

The Simpson's method of disc (SMOD) evolves tracing of LV internal chambers and computerised calculations treating the ventricles as a stack of discs. In this method volume of each disc was calculated and summated for the total LV volume. This method shows best correlation with actual left ventricular volume in the diseased heart and appears relatively unaffected by changes in the ventricular geometry (Tidholm *et al.*, 1997) . The present study involved SMOD for calculating EF. Therefore, reliability of this data is more, although several studies in dogs showed a high correlation between M-mode derived volumes and cardiac output by using Teicholz method in normal dogs, its reliability in diseased heart remains questionable because of the altered ventricular geometry

Highly significant increase in the mean ± S.E values of LVIDd, LVIDs, RVIDs and EPSS were noticed in DCM dogs (Figure-17) compared to control given in Table 3. Significant increase in the mean ± S.E values of RVIDd was noticed in DCM dogs compared to control. Highly significant decrease in the mean ± S.E values of IVSs, LVPWs, FS and IVS FT were noticed in DCM dogs compared to control. Significant decrease in the mean ± S.E values of LVPW FT was noticed in DCM dogs compared to control. No significant difference in the mean ± S.E values IVSd and LVPWd were noticed in DCM dogs compared to control.

Highly significant increase in the mean ± S.E values of LA peak E-velocity, RA peak E-velocity and TV peak E-velocity were noticed in DCM dogs(Figure-18) compared to control are given Table 3. Significant increase in the mean ± S.E values of MV peak E-velocity was in DCM dogs compared to control. Highly significant decrease in the mean ± S.E values of Ao velocity, PA velocity, LV OT velocity and RV OT velocity were noticed in DCM dogs compared to control. MV and TV regurgitant velocity were noticed only in DCM dogs.

Tidholm and Jonsson (1996) in a study of 37 Newfoundland dogs with DCM recorded left ventricular hypo kinesis in 100 per cent of cases with FS ranging from 5 to 22 per cent, left ventricular end diastolic diameter (LVIDd) ranged from 5.0 to 8.4cm and left ventricular end systolic diameter (LVIDs) ranged from 4.3 to 8.0 cm. This is in agreement with the present study. In the present study increased left ventricular dimensions in end diastole, end systole, thinning of interventricular septum and thinning of left ventricular posterior wall in diastole with reduced thickening of interventricular septum and left ventricular posterior wall during systole were observed. These findings concur with Vollmar (1999) who in a study of DCM in Irish wolfhounds reported similar findings. The presence or absence of left ventricular volume overload is determined from diastolic dimensions. This measurement reflect maximum ventricular filling when the heart is relaxed. Systolic dimensions are a reflector of systolic function in a heart and should not be used to assess the presence or absence of dilation. The same principle applies to wall and septal thickness measurements. The presence or absence of hypertrophy can be determined from diastolic measurement of thickness. Systolic measurements of wall thickness are a reflection of systolic function so reduced thickness during systole may simply reflect decreased function (Boon, 1998).

In the present study there was highly significant increase in EPSS (1.92±0.09cm) in DCM dogs compared to control. This finding concurred with several authors (McEwan *et al.*,2000 ; Vollmar, 1999 ; Vollmar , 2000; Calvert and Brown,1986) opined that EPSS was a most sensitive and specific criteria for the recognition of early cardiomyopathy. McEwan *et al.*,(2003) proposed increased mitral valve EPSS as one among the guidelines for the diagnosis of DCM.

Boon (1998) was of opinion that E-point to septal separation is one of the consistent and popular mitral and mitral insufficiencies. This correlation to ejection fraction is based on the fact that flow into the ventricle is equal to flow leaving ventricle. Therefore in DCM presence of high end diastolic left ventricular pressures reduce flow from left atrium to left ventricle due to reduced ventricular complaince and consequently flow out of left ventricle is also reduced. EPSS accurately separates normal from abnormal left ventricular function regardless of left ventricular size when dilatation is present. Hence EPSS is also valid for assessing left ventricular function in the presence of abnormal septal motion.

Highly significant decreased in the fraction shortening was observed in DCM dogs compared to control. This findings is in agreement with Atkins and Snyder (1992); Monnet *et al.* (1995); Tidholm and Jonsson (1996); McEwan *et al*.(2000); McEwan *et al*.(2003) and Borgarelli *et al.* (2006).

Kittleson (1998) categorised the severity of DCM based on M-mode derived fraction shortening as mild, moderate and severe therefore in the present study DCM can be categorised as severe since the mean FS was 13.45±0.73per cent.

Tidholom and Jonsson (1996) in a study of 37 Newfoundland dogs with DCM recorded left ventricular hypo kinesis in 100 per cent of cases with FS ranging from 5 to 22 per cent which concurs with the present study. It is important to remember that fractional shortening is not a measure of contractility but is a measure of function. The three conditions that affect the fraction shortening the most are preload, after load and contractility. Each one of these may individually or together affect the FS. When a low fractional shortening is calculated, it may be secondary to poor preload, increased after load or decreased contractility. To differentiate between these factors left ventricular end diastolic dimension measurement and blood pressure measurement are important. When increased left ventricular diastolic size is present and normal blood pressure is measured then reduced preload and increased afterload can be ruled out. Therefore measurement of blood pressure and LVIDd remains as a deciding factor to say that poor contractility is present.

Pulse wave Doppler

Highly significant increase in the LA peak E-velocity, RA peak E-velocity and TV peak E-velocity were noticed in DCM dogs compared to control. Significant increase in the MV peak E-velocity was in DCM dogs compared to control. Highly significant decrease in the Ao velocity, PA velocity, LV OT velocity and RV OT velocity were noticed in DCM dogs compared to control. MV and TV valve regurgitation were observed in DCM dogs.

In the present study for mitral valve, tricuspid valve, LA and RA only the peak E-velocities were taken into account. This is because in normal animals, peak E-velocity represents rapid ventricular filling and peak A represents secondary atrial contractions. Where in DCM cases, rapid heart rate will cause two filling phases to overlap resulting in super imposed waveforms, this overlap will start to appear at heart rates approaching 125 beats per minute and complete loss of separation always will be present when the heart rate exceeds 200 beats per minute. In the present study the average heart rate was 163.33±3.29 bpm and therefore only the peak E-velocity were recorded.

Highly significant increase in tricuspid valve velocity (2.70±0.04 m/sec), LA velocity (1.18±0.07m/sec), RA velocity (1.06±0.08m/sec) and significant increase in mitral valve velocity (1.15±0.07m/sec) were appreciated in the present study. The increased E-velocity may be secondary to increased atrial pressure or volume associated with insufficiency of the valves concerned.

The average regurgitant velocity recorded in the present study at mitral and tricuspid valves were 3.21±0.03 m/sec and 2.70±0.04 m/sec respectively. This regurgitant velocity may be attributed to mild to moderate regurgitation at the valves because of the altered geometry of the ventricles due to dilatation and pulmonary hypertension.

Highly significant decrease observed in the velocities of all out flow valves i.e. aorta (0.78±0.03m/sec), pulmonary artery (0.54±0.02m/sec), LVOT (0.80±0.03m/sec), and RVOT (0.55±0.02m/sec) were noticed in DCM dogs compared to control. These findings may be attributed to severe systolic failure in the DCM affected dogs.

Colour flow Doppler echocardiography

In control group normal flow pattern was observed in LA, RA, Ao, PA, MV, TV, LV OT and RV OT. In DCM group(Figure-19) no change in the flow pattern was observed across Ao and PA in any of the dogs, where as normal flow pattern across MV and TV were observed in only 33 per cent (10/30) of dogs. Mitral valve regurgitation was noticed in 67 per cent (20/30) of dogs which include mild regurgitation in 44 per cent (13/30) of dogs and moderate regurgitation in 23 per cent (7/30) of dogs. Tricuspid valve regurgitation was noticed in 40 per cent (12/30) of dogs which include mild regurgitation in 23 per cent (7/30) of dogs and moderate regurgitation in 17 per cent (5/30) of dogs. Both mitral and tricuspid valve regurgitation was noticed in 40 per cent (12/30) of dogs.

Boon (1998) assessed regurgitation semi quantitatively by measuring the size of color flow set within the atria for atrio-ventricular (AV) valves. A jet occupying less than 20 of the atrium was considered to represent mild insufficiency and jet that occupied 20-50 per cent of atria was considered to represent moderate regurgitation. In the present study only mild to moderate regurgitation was appreciated as the jet was occupying less than 50 per cent of the atrial area. This regurgitation may be attributed to abnormal dilation of the chambers leading to altered geometry and consequent leak in the AV valves and pulmonary hypertension.

Post mortem examination

Post-mortem examination was performed in 3 dogs that were died during the study period. Grossly heart was enlarged, globular, and flabby in appearance with rounded apex due to generalized ventricular dilatation (Figure20). On transverse biventricular slice of heart the marked dilatation of lumen, attenuation of papillary muscles and thinning of free walls of ventricles were observed. The histopathology(Figure-21) of left ventricular myocardium showed attenuated wavy fibre type of myofibers with thinner, well separated and wavy appearance were observed in two dogs and fatty infiltration-degenerative type with vacuolar or fatty degeneration of myofibers, marked vacuolative degeneration were observed in one dog.

Grossly heart was enlarged globular, flabby in appearance with rounded apex due to generalized ventricular dilatation. On transverse biventricular slice of heart the marked dilatation of lumen, attenuation of papillary muscles and thinning of free walls of ventricles were observed. These findings were in agreement with Tidholom and Jonsson (1996) ; Sisson *et al*., (1999). They reported dilation of all four cardiac chambers or predominant dilation of left chambers. Myocardial eccentricity was evident with decreased ratio of left ventricular wall to chamber diameter as reported by several authors (Sisson and Thomas, 1995; Sisson *et al.*,1999). In histopathology of ventricular myocardium, attenuated way fibre type of myofibres with thinner, well separated and wavy appearance was identified in two dogs and the other dog showed fatty infiltration degenerative type with marked vacuolated degeneration. Tidholm and Jonsson (2005) and Dambach *et al.*, (1999) reported attenuated wavy fibre as the major histological findings in dogs with DCM which concurs with the present findings since two dogs had attenuated wavy fibres. Tidholm and Jonsson (2005) stated that histological findings in dogs with clinically diagnosed DCM revealed two distinct form of DCM: 1.Cardiomyopathy of Boxers and Doberman pinchers showing infiltrative degenerative type 2. Many medium and giant sized breed including Boxers and Doberman pinchers showing attenuated wavy fibres. The present study is having both the types of histological changes therefore it is in agreement with the above authors

Tables 1- Mean ± S.E of haematological values and biochemical values in control and DCM dogs

Parameters	Control Mean±S.E	DCM Mean±S.E	T-value
Hb(g/ml)	11.60±0.19	10.77±0.33	0.031*
PCV (%)	33.98±0.79	30.78±1.01	0.016*
RBC (million/ cumm)	5.14±0.15	4.68±0.15	0.034*
WBC (cells/cumm)	14,963.3±1,129.8	21,126.6±1,729.5	0.004**
Platelet	270,300.0±18,431.5	218,966.6±21,702.2	0.077^{NS}
Absolute neutrophils(cells/cumm)	11,830.20±975.57	17,580.50±1,594.41	0.003**
Absolute lymphocytes(cells/cumm)	2,822.06±209.81	2,686.36±217.10	0.655^{NS}
Absolute monocytes(cells/cumm)	224.66±27.26	479.50±141.43	0.082^{NS}
BUN(mg/dl)	16.43±0.74	28.89±3.21	0.00**
Creatinine (mg/dl)	1.44±0.04	1.80±0.10	0.00**
ALT (IU/l)	80.01±1.27	113.07±24.10	0.17^{NS}
SAP (IU/l)	131.19±7.04	148.41±12.27	0.23^{NS}

TP (g/dl)	6.53±0.09	6.16±0.09	0.01*
Albumin (g/dl)	2.91±0.07	2.91±0.07	1.00 NS
Sodium (mmol/l)	116.6±3.9	122.8±4.6	1.03 NS
Potassium (mmol/l)	4.53±0.17	5.24±0.25	0.03*
Calcium (mg/dl)	11.87±0.35	12.18±0.38	0.55 NS
Phosphorus (mg/dl)	5.85±0.20	6.46±0.30	0.10 NS

** - Statistically highly significant (P<0.01)
* - Statistically significant (P<0.05)
NS - Statistically non-significant (P>0.05)

Table 2: Mean ± S.E of Radiographic Vertebral heart score and electrocardiographic values in control and DCM dogs

Parameters	Control Mean ± S.E	DCM Mean ± S.E	T-value
Vertebral heart score	10.78±0.03	12.51±0.14	0.00**
P-amplitude	0.16±0.01	0.18±0.01	0.46 NS
P-duration	0.03±0.00	0.03±0.00	0.35 NS
PR-interval	0.09±0.00	0.09±0.00	0.88 NS
QRS- duration	0.03±0.00	0.03±0.00	0.35 NS
R-amplitude	1.65±0.06	1.75±0.13	0.51 NS
QT- interval	0.20±0.01	0.19±0.02	0.35 NS
Heart rate	113.33±3.15	163.33±3.29	0.00*

NS - Statistically non-significant (P>0.05)

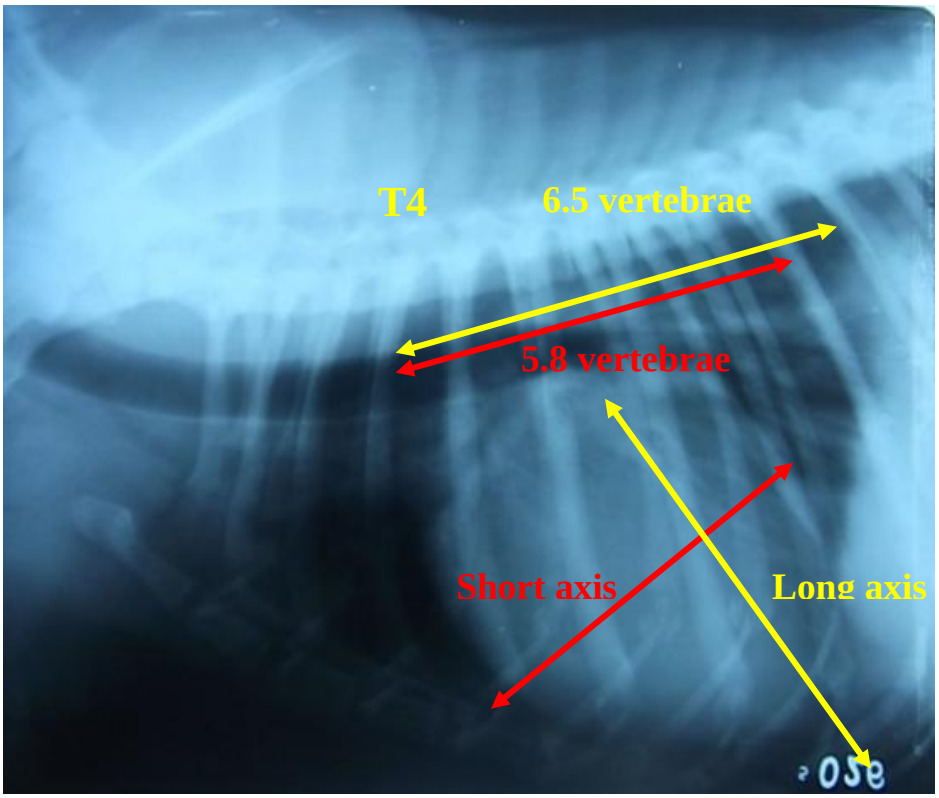
Table-3: Mean±S.E of Two dimensional, M-mode, Pulsed wave Doppler echocardiography values in control and DCM dogs

Parameters	Control Mean±S.E	DCM Mean±S.E	T-value
LA cm	2.34±0.04	4.09±0.05	0.00**
Ao cm	2.28±0.04	2.08±0.05	0.00**
LA/Ao	1.02±0.00	2.01±0.06	0.00**
EDV ml	53.06±0.68	95.16±1.97	0.00**
ESV ml	24.96±0.36	67.30±2.36	0.00**
SVml	28.10±0.63	27.86±2.22	0.92 NS
EF (%)	52.81±0.73	29.11±2.13	0.00**
LVIDd cm	3.99±0.05	5.84±0.13	0.00**
LVIDs cm	2.60±0.04	5.05±0.12	0.00**
RVIDd cm	0.94±0.05	1.26±0.11	0.01*
RVIDs cm	0.80±0.04	1.24±0.09	0.00**
IVSd cm	0.76±0.02	0.70±0.03	0.16 NS
IVS s cm	0.97±0.03	0.81±0.04	0.00**
LVPWd cm	0.90±0.04	0.79±0.03	0.06 NS
LVPWs cm	1.23±0.04	0.97±0.03	0.00**
FS%	34.68±0.88	13.45±0.73	0.00**
EPSS	0.80±0.01	1.92±0.09	0.00**
IVS FT%	27.20±1.58	15.99±2.38	0.00**
LVPW FT%	39.91±3.26	26.02±5.58	0.03*
LA Peak E-velocity (m/sec)	0.96±0.04	1.18±0.07	0.00**
RA Peak E-velocity (m/sec)	0.92±0.03	1.06±0.08	0.00**
Ao (m/sec)	1.18±0.02	0.78±0.03	0.00**

PA (m/sec)	0.99±0.01	0.54±0.02	0.00**
TV Peak E-velocity (m/sec)	0.90±0.03	1.12±0.06	0.00**
MV regurgitant velocity(m/sec)	Nil	3.21±0.03	Nil
TV regurgitant velocity(m/sec)	Nil	2.70±0.04	Nil
LV OT (m/sec)	0.98±0.03	0.80±0.03	0.00**
RV OT (m/sec)	0.94±0.02	0.55±0.02	0.00**

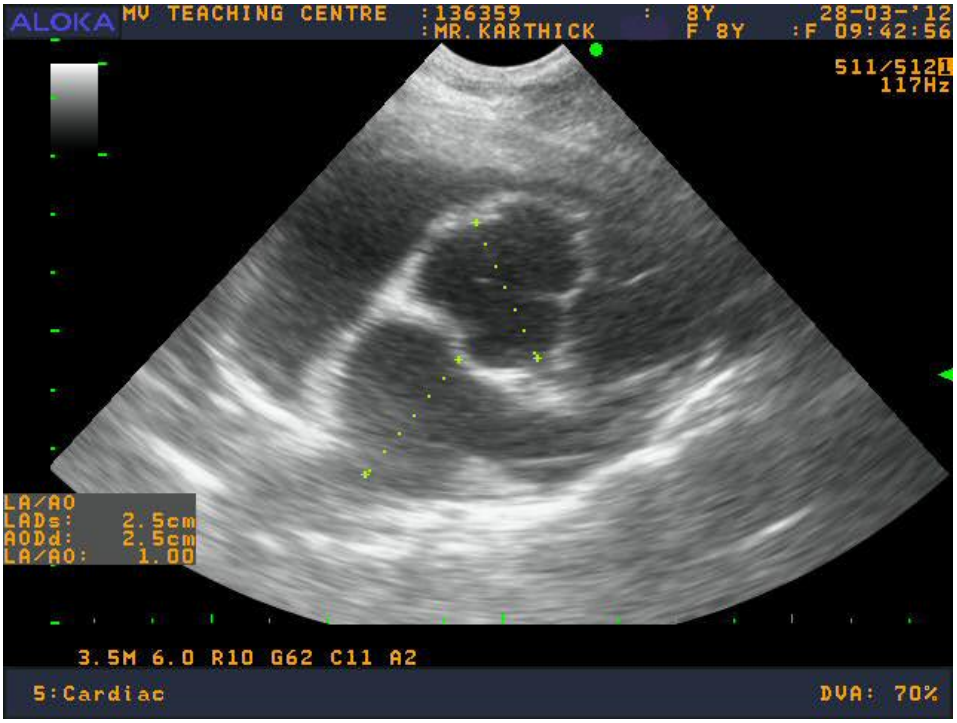
** - Statistically highly significant (P<0.01)
* - Statistically significant (P<0.05)
NS - Statistically non-significant (P>0.05)

Figure:1 Thoracic Radiography measuring Vertebral Heart Score

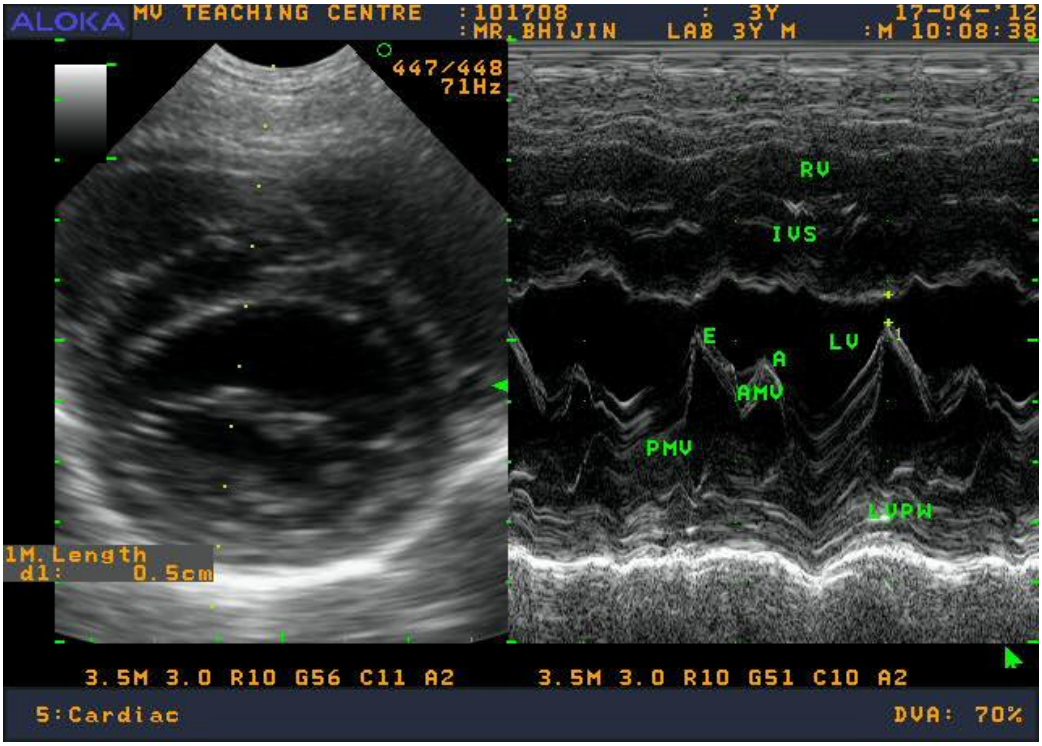


VHS = 6.5(long) + 5.8(short) = 12.3

Figure:2 Two dimensional echocardiography in control dogs



2a. Measurement of Left Atrium / Aorta ratio



2b. EPSS measurement at the level of Mitral valve

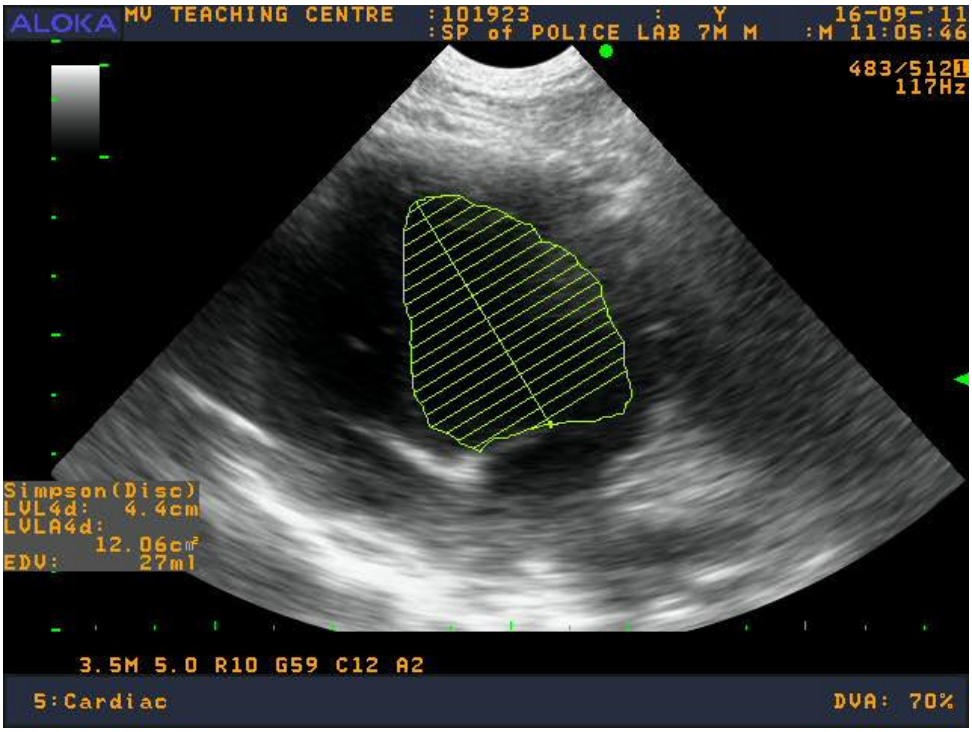


Figure 3a. Simpson's method of Disc(SMOD) measuring Ejection Fraction SMOD at diastole in left apical four chamber view

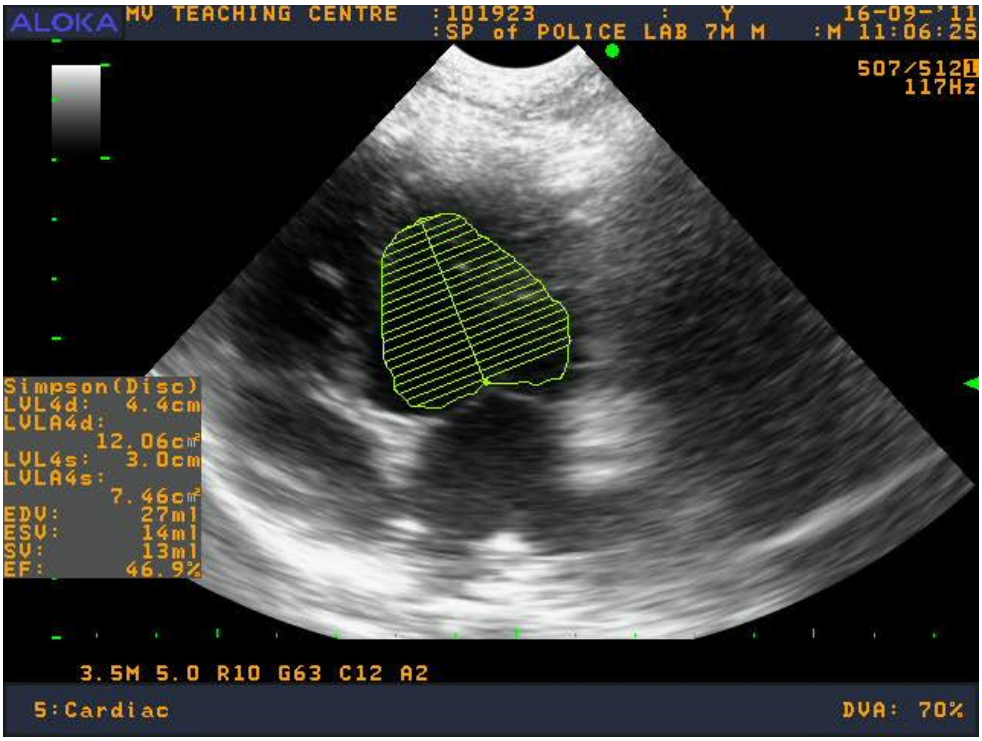
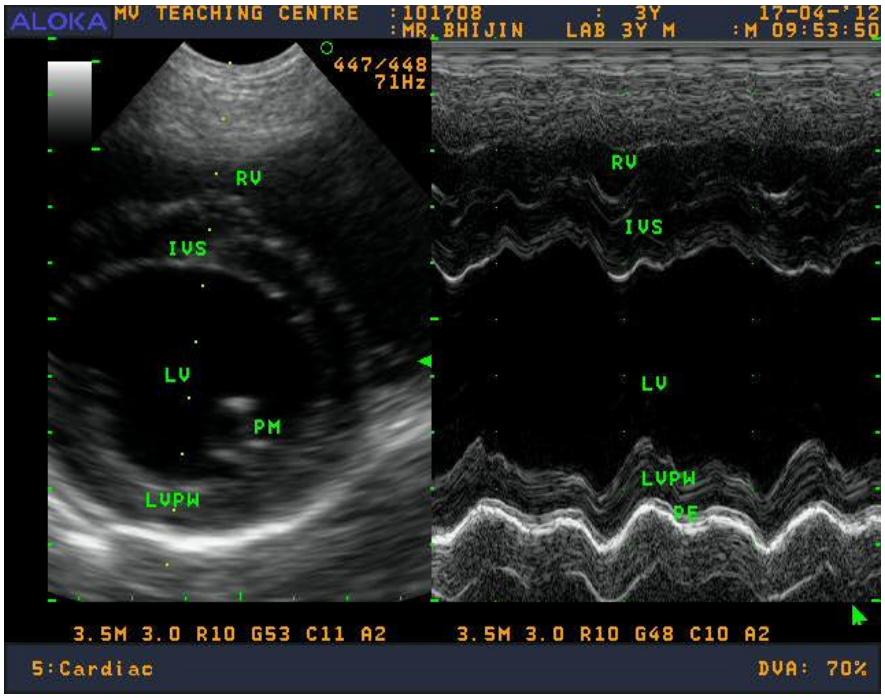


Figure 3b. SMOD at systole in left apical four chamber view

Figure:4 M-mode echocardiography in control dogs



4a. M-mode at the level of Papillary muscles

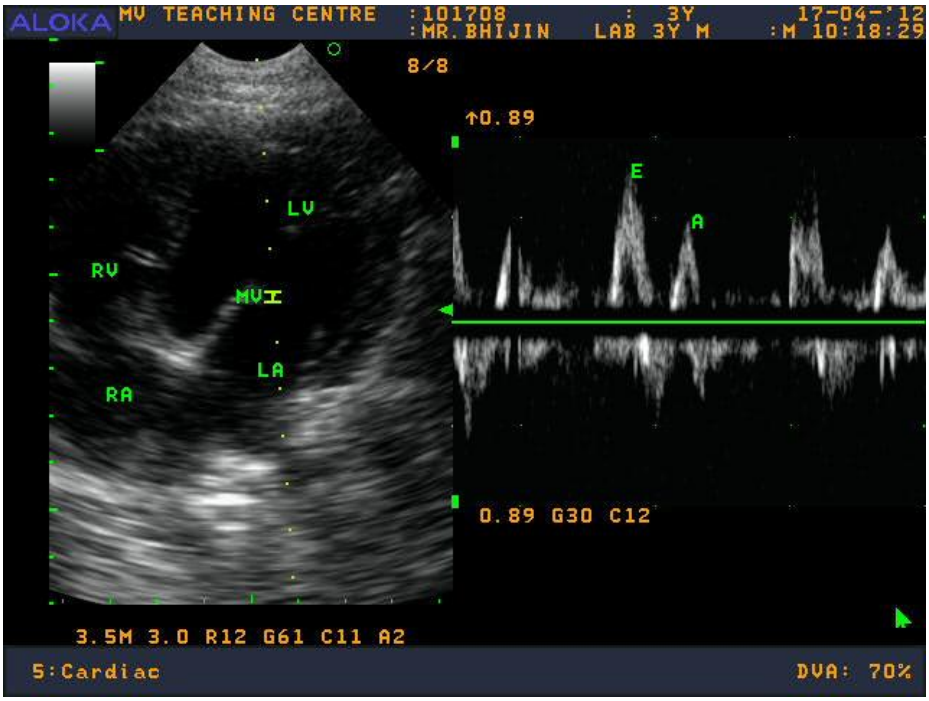


Figure – 5: Pulsed wave Doppler echocardiography measuring Mitral valve velocity

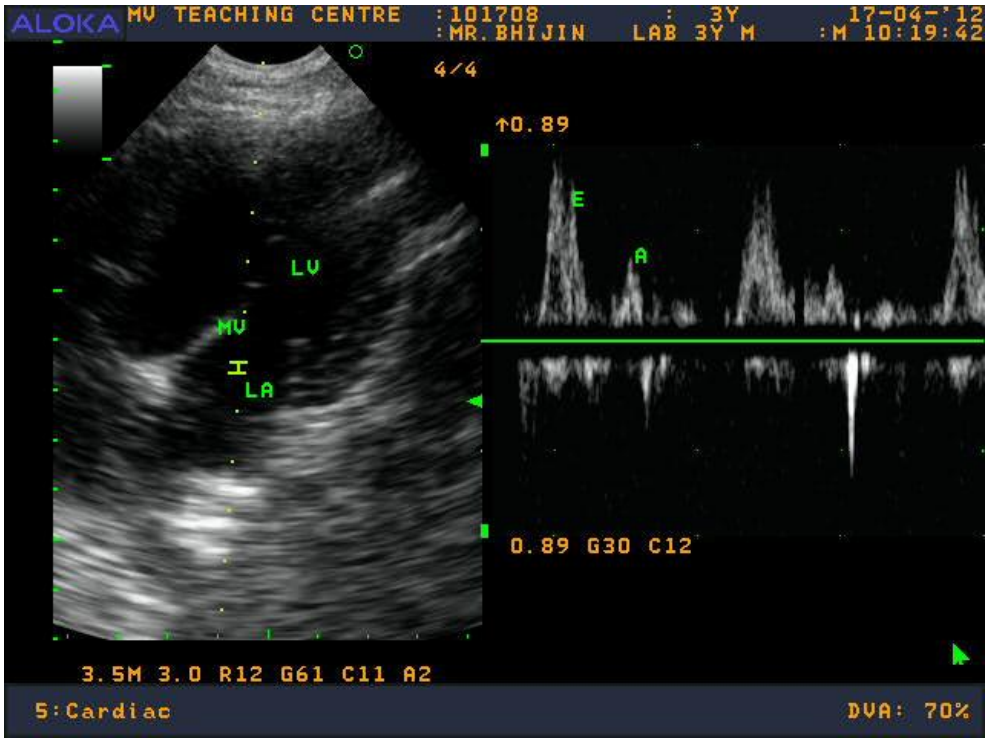


Figure 6. Pulsed wave Doppler echocardiography measuring Left atrium velocity

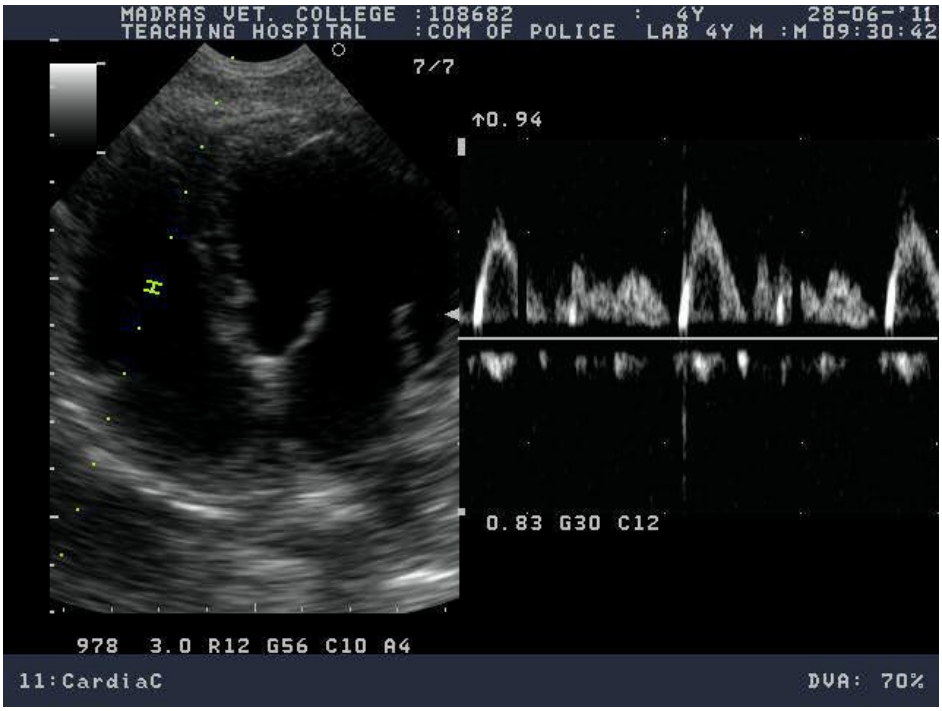


Figure 7. Pulsed wave Doppler echocardiography measuring Tricuspid valve velocity

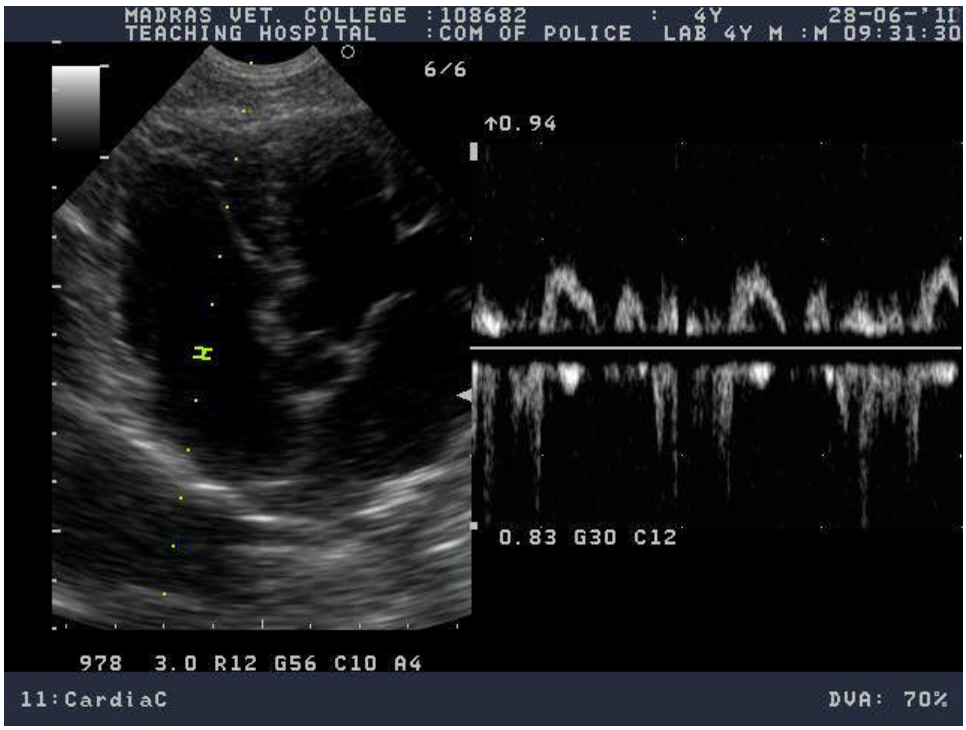


Figure -8 . Pulsed wave Doppler echocardiography measuring Right atrium velocity

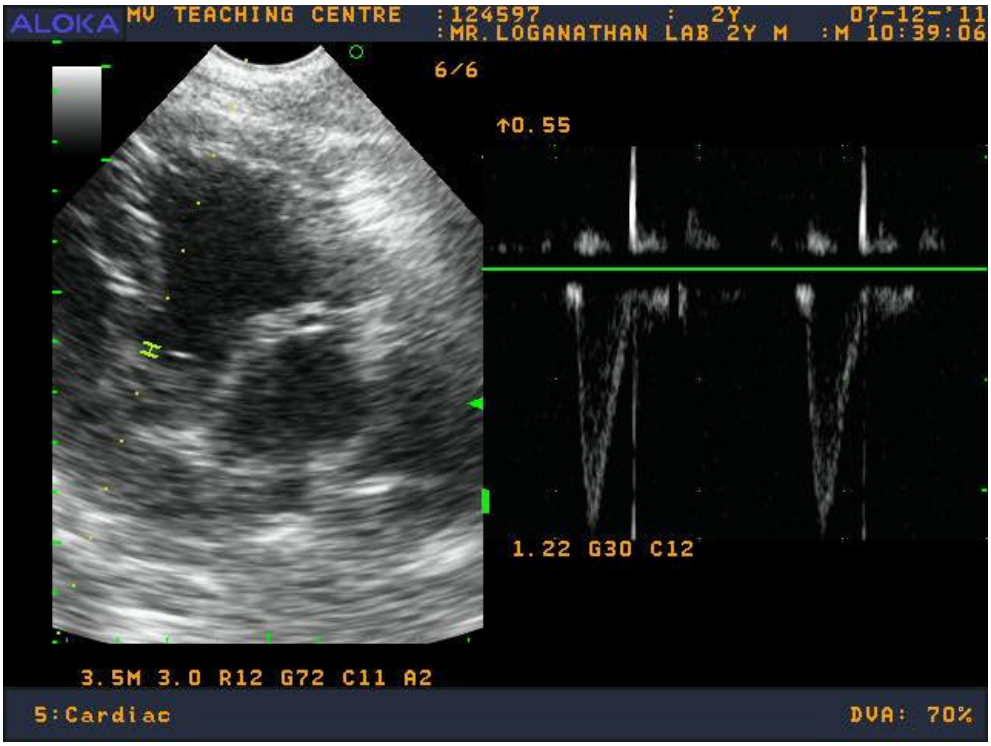


Figure- 9. Pulsed wave Doppler echocardiography measuring aortic velocity

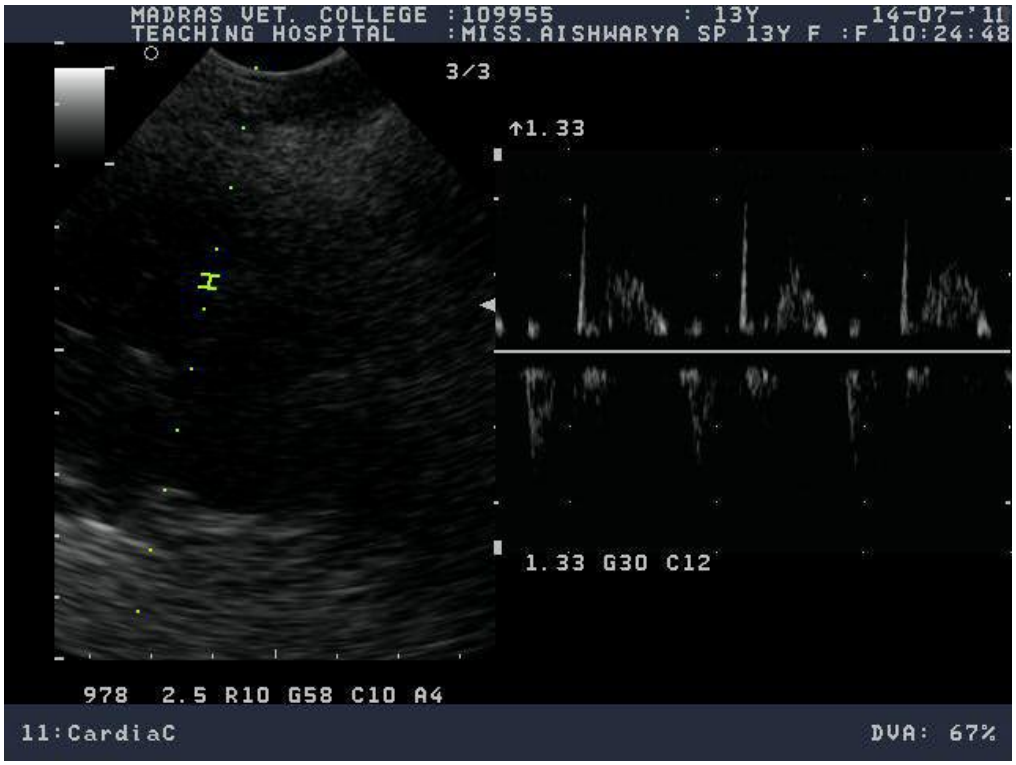


Figure - 10. Pulsed wave Doppler echocardiography measuring Left Ventricular outflow velocity

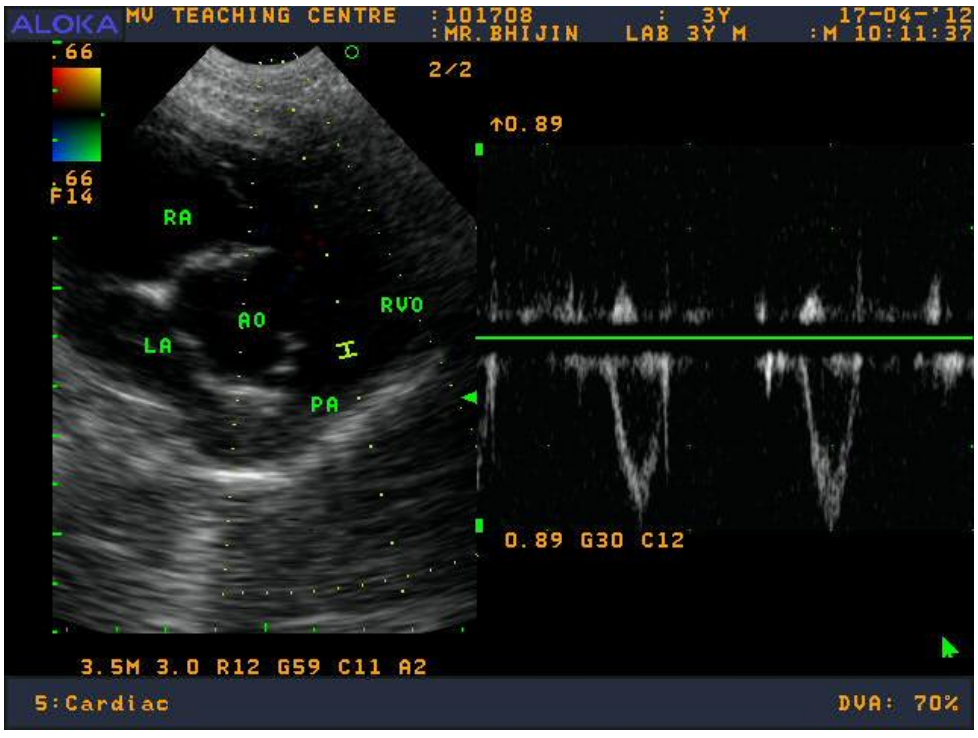


Figure- 11. Pulsed wave Doppler echocardiography measuring pulmonary artery velocity

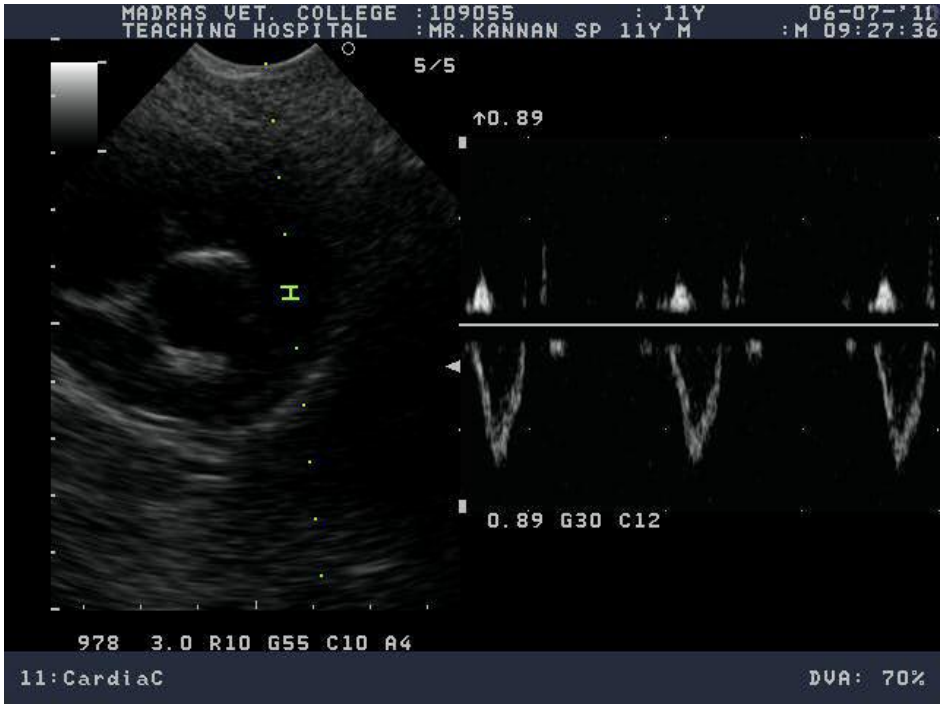


Figure 12. Pulsed wave Doppler echocardiography measuring Right Ventricular outflow velocity

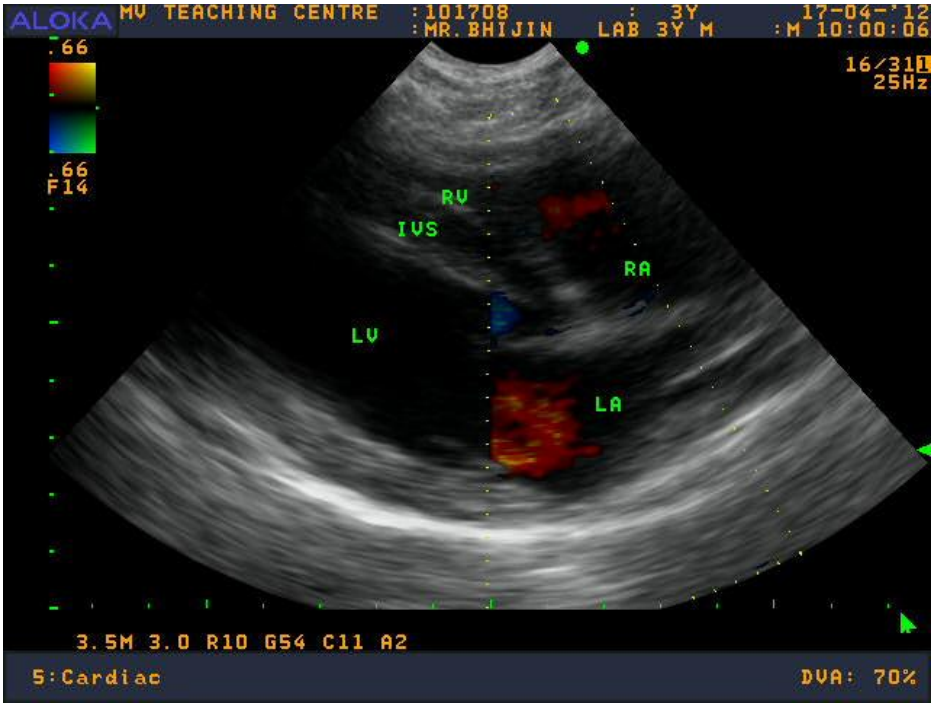


Figure- 13 Normal color flow across Mitral and Tricuspid valves at right parasternal long

axis view

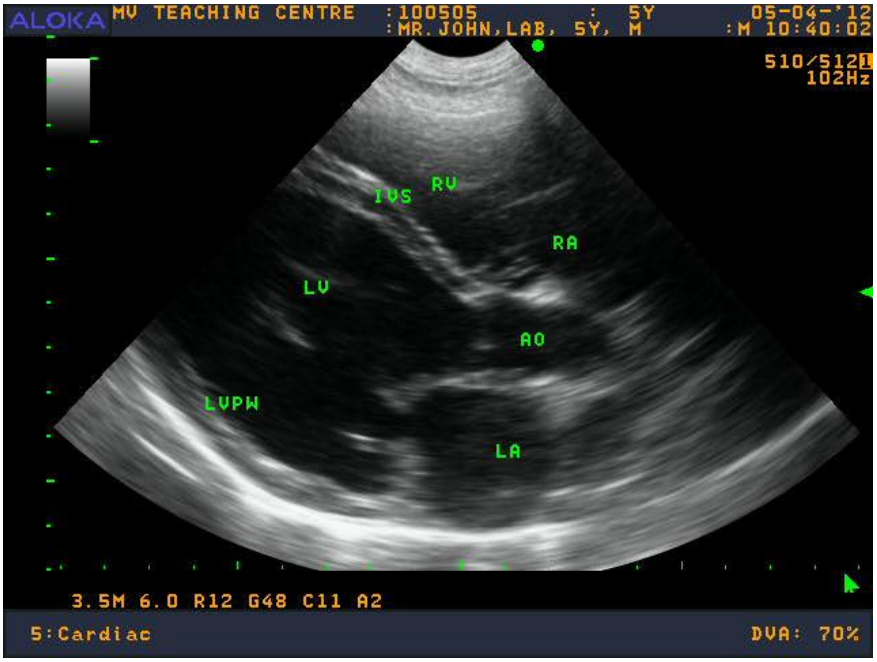


Figure 14: . Left Ventricular dilatation and sphericity in parasternal long axis 5 chamber view

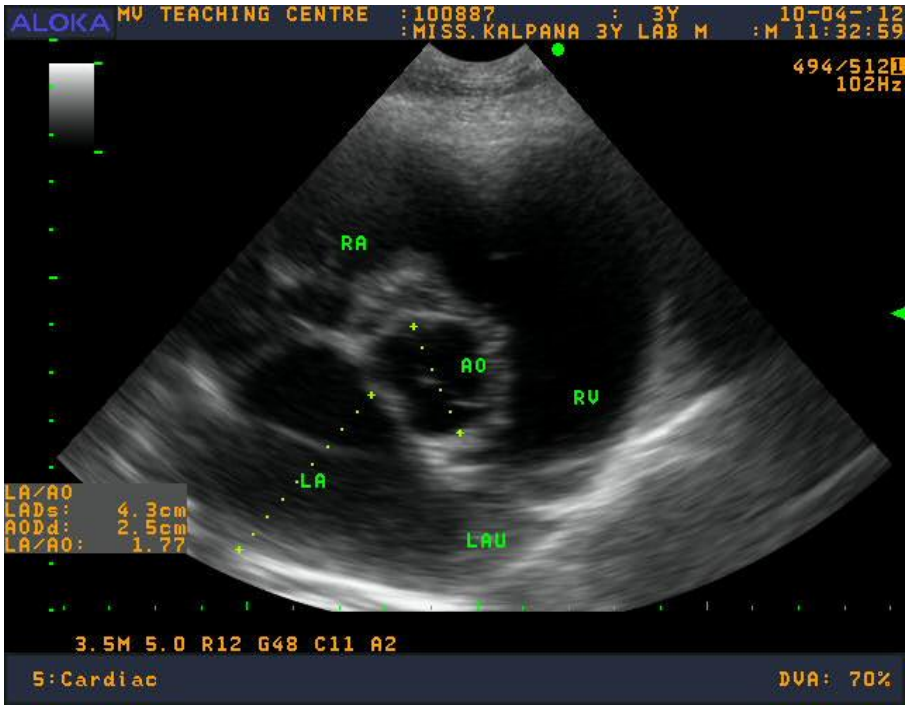
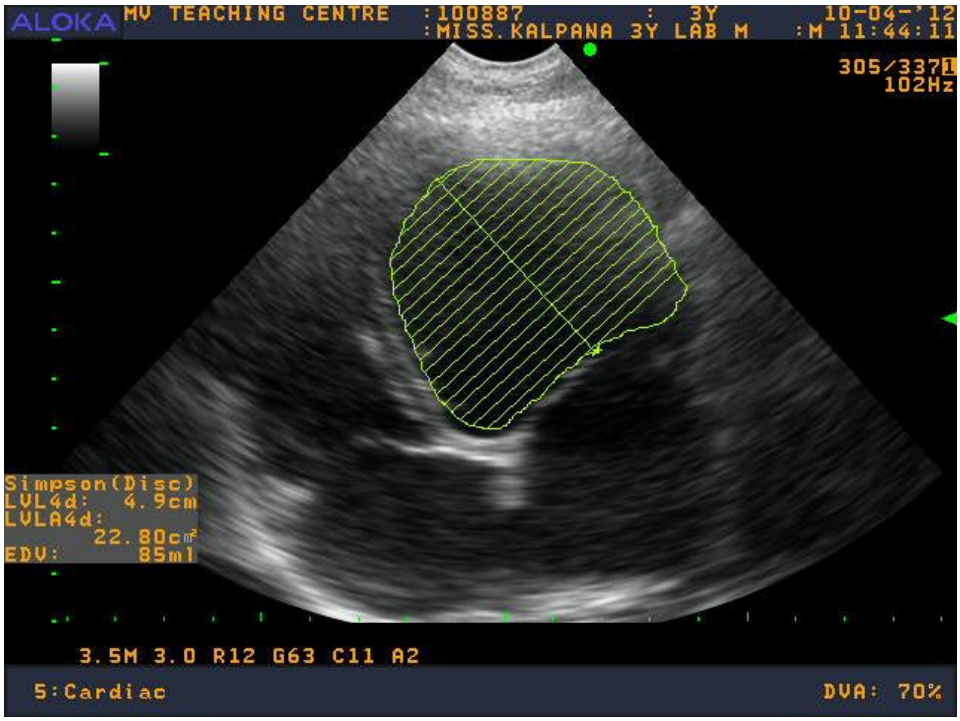
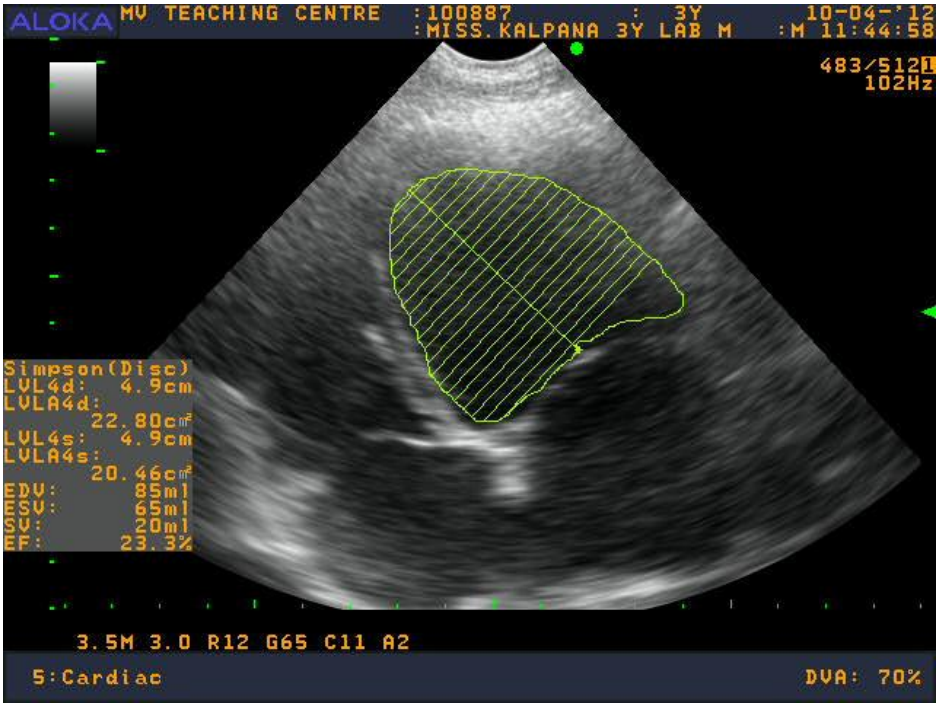


Figure 15 . Dilated Left atrium at the right parasternal short axis view

Figure 16 : Simpson's method of Disc in DCM affected Labrador retrievers

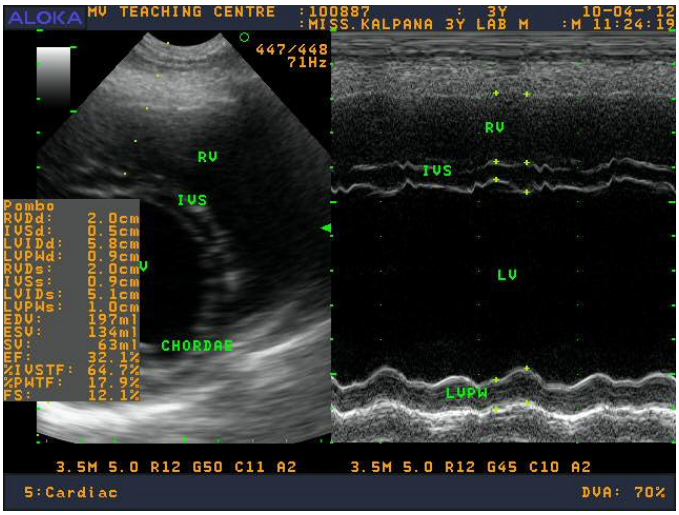


16a. SMOD at diastole in left apical four chamber view

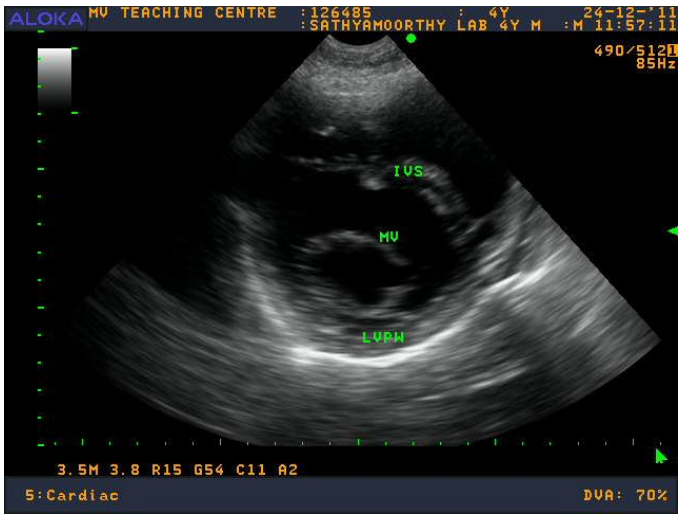


16b. SMOD at systole in left apical four chamber view

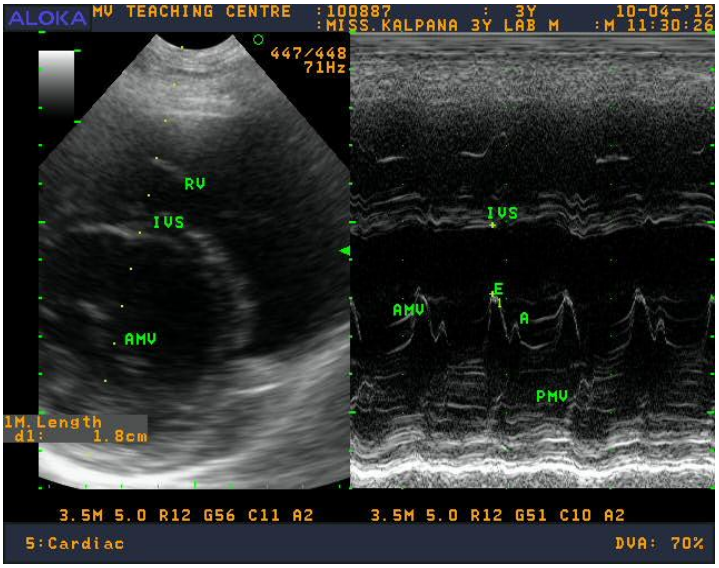
Figure 17. M-mode echocardiography in DCM affected Labrador retrievers



17a. M-mode measurements of Left ventricle at the level of papillary muscle

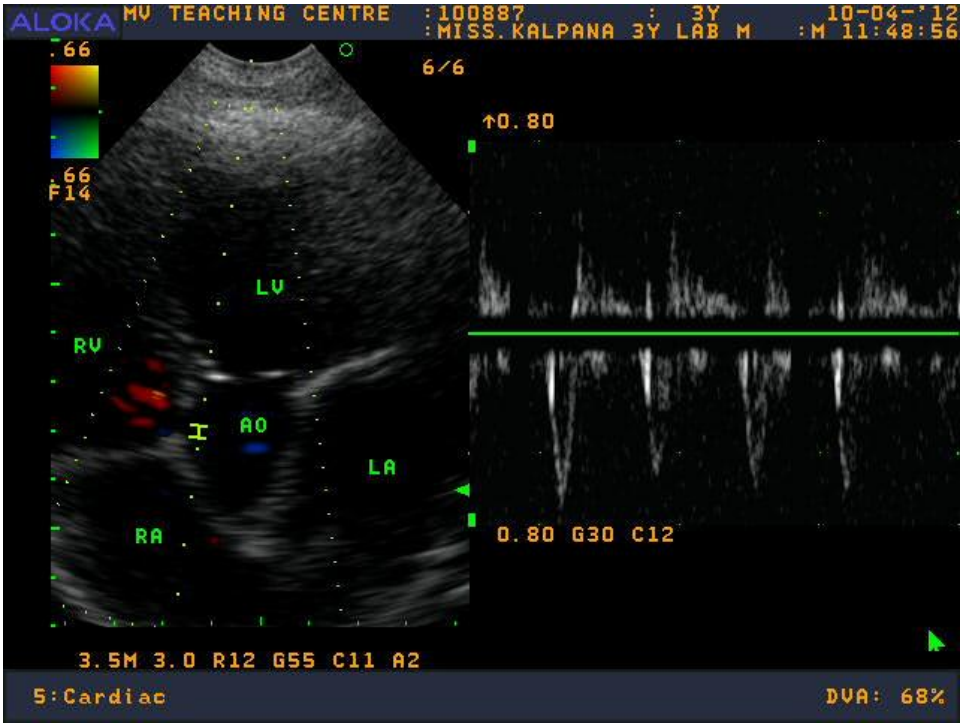


17b. Right parasternal short axis view showing Anterior Mitral valve and Posterior Mitral valve as fish mouth

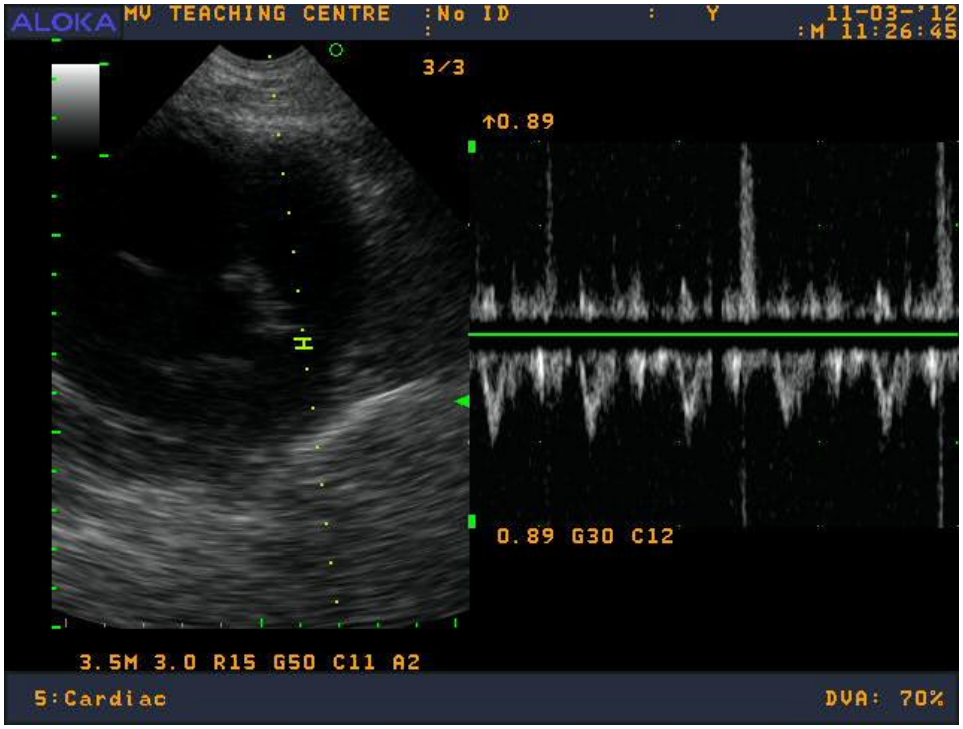


17c. Measuring of EPSS at the level of Mitral valve

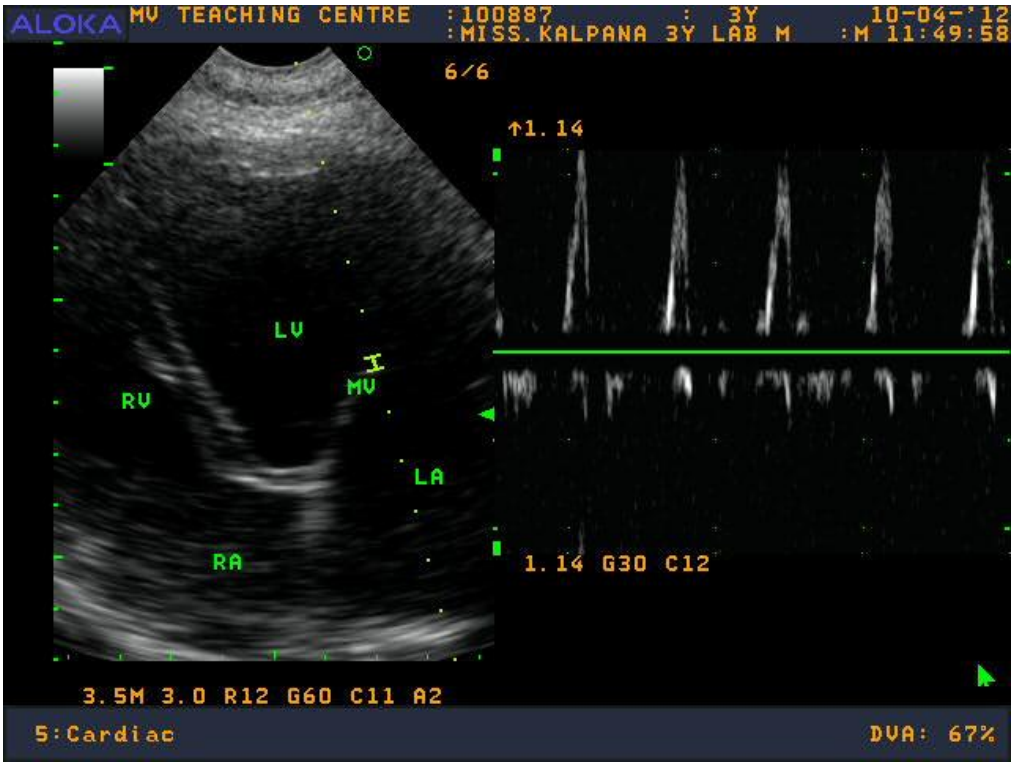
Figure 18. Pulsed wave Doppler echocardiography in DCM affected Labrador retrievers



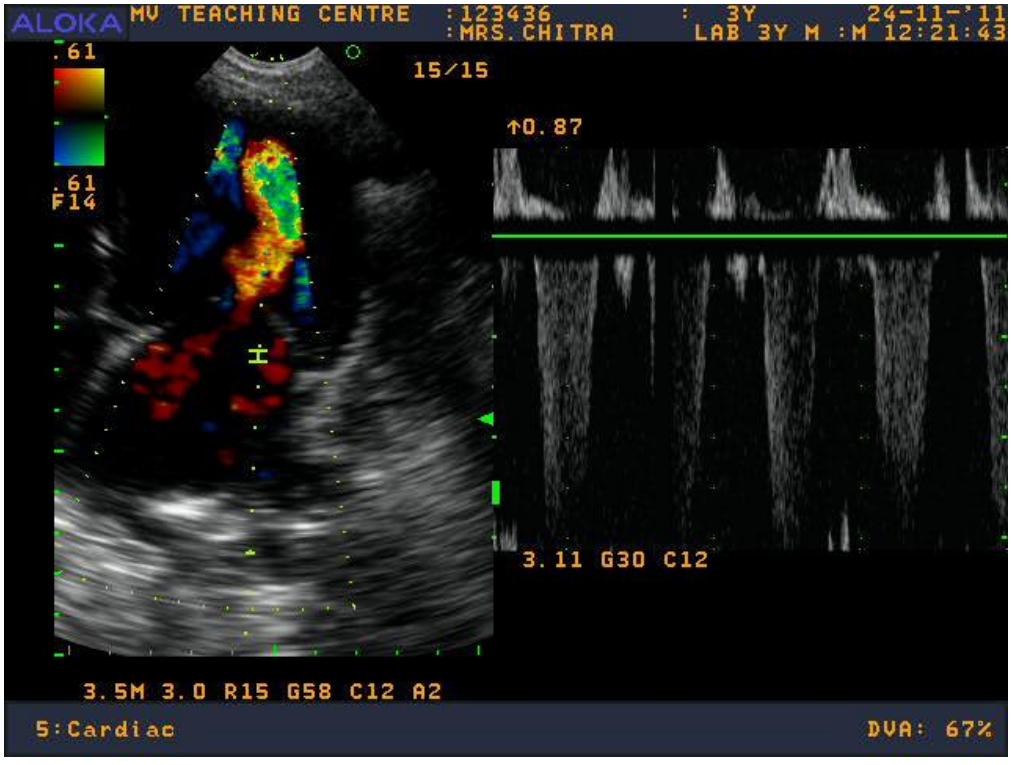
18a. Reduced Aortic velocity in left apical five chamber view



18b. Reduced Pulmonary artery velocity at right parasternal short axis view

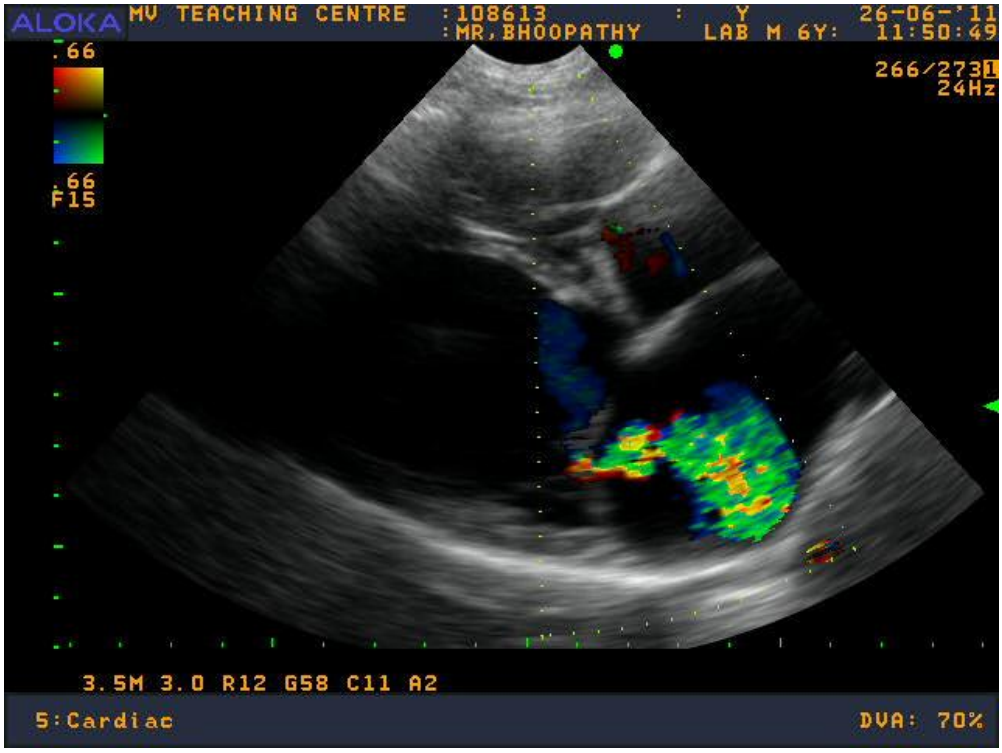


18c: Increased Mitral valve and absence of A-point

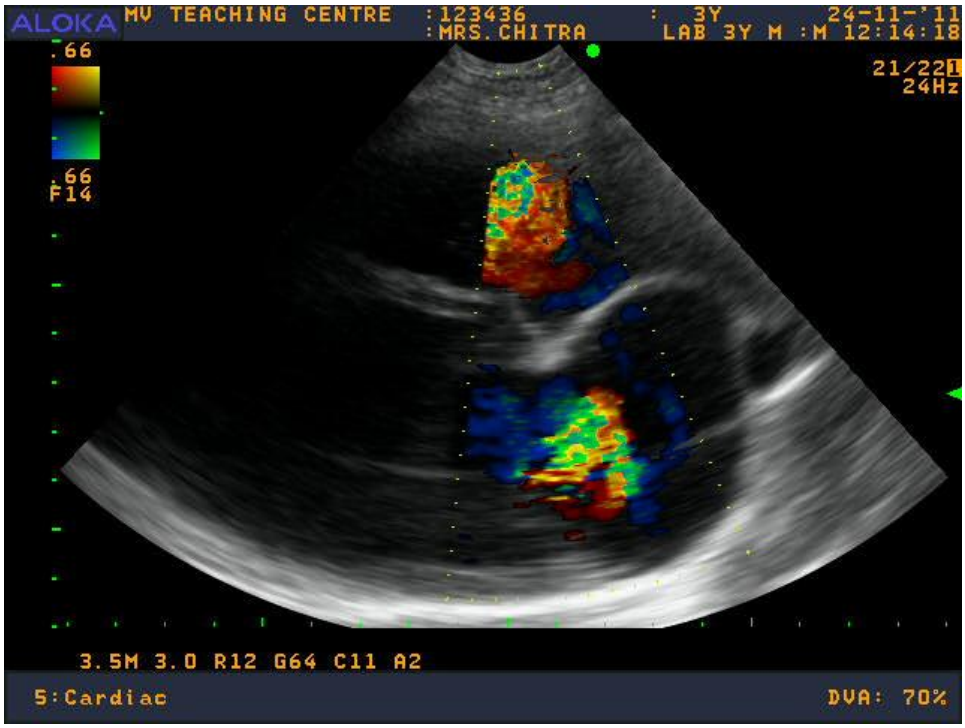


18d . Regurtitant Mitral valve velocity

Figure 19. Color flow Doppler echocardiography in DCM affected Labrador retrievers

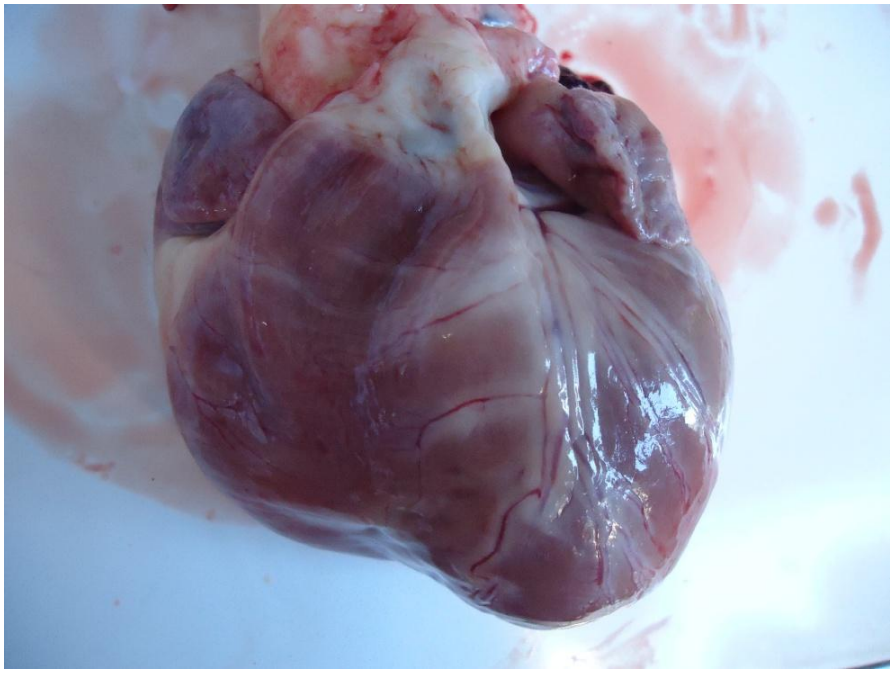


19a. Mitral valve regurgitation

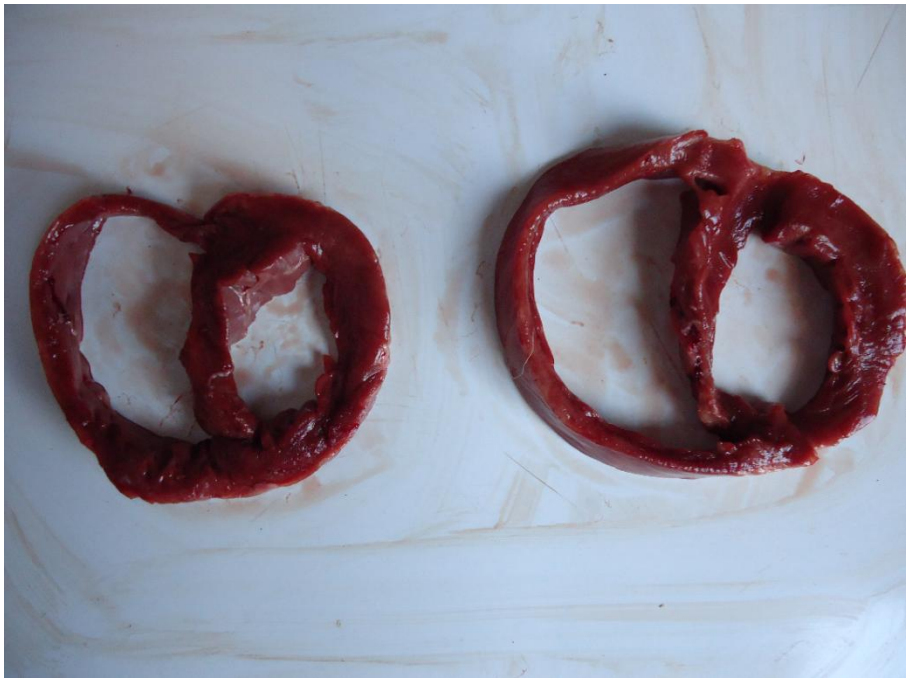


19b. Mitral and Tricuspid valve regurgitation

Figure 20. Post-mortem findings in DCM affected Labrador retrievers

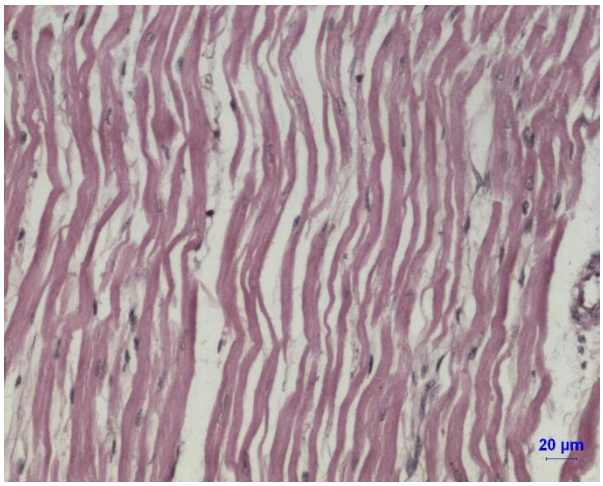


20a. Rounded, flabby and dilated heart

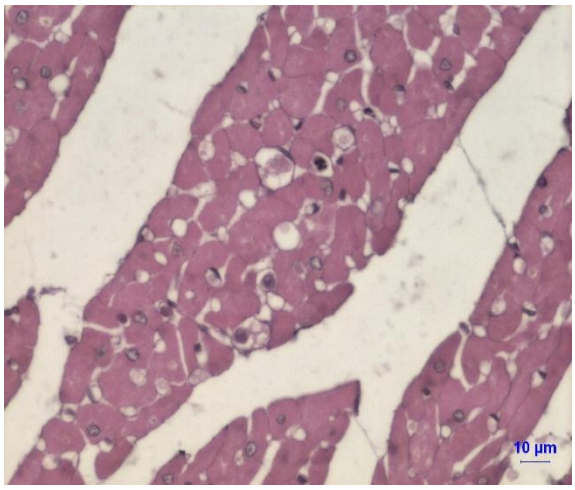


20b. Biventricular dilatation and wall thinning at different levels

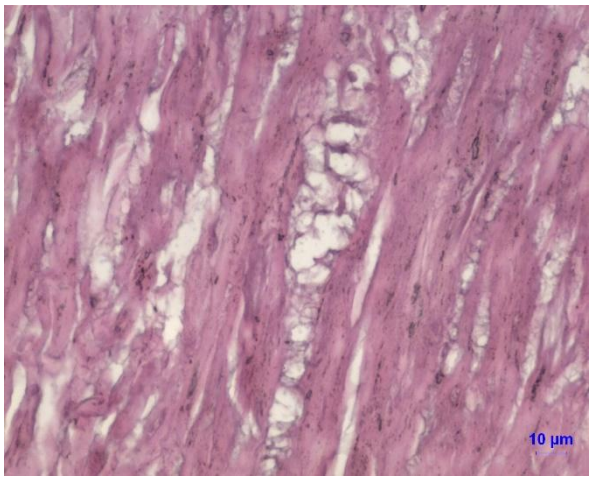
Figure 21. Histopathological findings in DCM affected Labrador retrievers



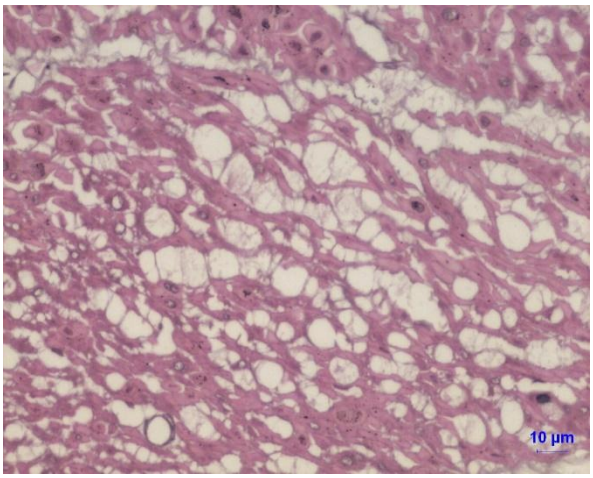
21a. Attenuated wavy fibre degeneration



21c. Fatty degeneration



21b. Myofibril degeneration



21d. Vaculative degeneration

Conclusion

It was concluded that major complaints in Labrador dogs with DCM were abdominal distension, exercise intolerance, weight loss and cough. The prominent physical examination findings were gallop rhythms, systolic murmurs, limb edema and weak femoral pulse. Radiography acts as an important aid in assessing pulmonary edema and the Heart Score was found to be good indicator of dilatation in Labrador retrievers. Although electrocardiography was not effective in diagnosing cardiomegaly, it remains as a sole aid in diagnosing arrhythmias. In echocardiography two dimensional derived volumes and ejection fraction were found to be more reliable in Dilated Cardiomyopathy of Labrador retrievers. M-mode derived chamber dimensions, E-point septal separation and Fractional shortening remains as a gold standard for diagnosing Dilated Cardiomyopathy in Labrador retrievers. Pulsed wave doppler and color flow doppler were useful in assessing velocity and flow pattern across valves.

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