

A detailed study of non-traumatic cardiac diseases in *Bubalus bubalis* (domestic water buffaloes)

Chetan Jajoria¹, Tarun Kumar², Neelesh Sindhu², Ricky Jhambh¹, Maneesh Sharma²,
Annu Yadav¹, Vinay Ganeshrao Joshi³, Dipin Chander Yadav⁴

Lala Lajpat Rai University of Veterinary and Animal Sciences, College of Veterinary Science,
¹Department of Veterinary Medicine, ²Department of Veterinary Clinical Complex,
³Department of Animal Biotechnology, ⁴Department of LPM (buffalo farm), Hisar, Haryana, India

Received April 30, 2025

Accepted June 15, 2026

Abstract

Domestic water buffaloes (*Bubalus bubalis*) are the backbone of Indian dairy industry, contributing more than 55% of total milk produced by Indian livestock. Non-traumatic cardiac affections in buffaloes are commonly reported but remain underdiagnosed and understudied. This study was carried out on 1,768 buffaloes over a period of 8 months. Of the total, 57 buffaloes were suspected for cardiac diseases. Clinical signs, haemato-biochemical changes, radiography, echocardiography and cardiac biomarkers were estimated and correlated to characterize cardiac affections in buffaloes. Twenty-six buffaloes were confirmed non-traumatic cardiac affected cases and characterized into three categories, namely, idiopathic congestive heart failure (CHF) (16), CHF due to dilated cardiomyopathy (DCM) (8), and constrictive pericarditis (2) based on characteristic echocardiographic findings. Haemato-biochemical examination revealed neutrophilic leukocytosis, hypoalbuminaemia, hypoproteinaemia, hypocalcaemia and increased lactate dehydrogenase (LDH). Bovine cTn-I was significantly increased in all cardiac categories compared to healthy control. *Streptococcus* spp., *Staphylococcus* spp., *E. coli* spp. and *Corynebacterium* spp. were predominant bacterial isolates present in infected pericardial fluid. This is India's first report of DCM and idiopathic CHF in buffaloes of non-traumatic origin characterized on the basis of echocardiographic indices. Bovine cardiac troponin I (cTn-I) can serve as a sensitive diagnostic biomarker for cardiac affections in buffaloes but lacks specificity in differentiating specific cardiac diseases.

Cardiac troponin-I, DCM, LDH, echocardiography, heart affections

Cardiac affections in bovines constitute a major health issue, resulting in substantial economic losses due to abrupt reductions in milk production, elevated treatment costs, and potential animal mortality. These economic impacts can be significantly mitigated through early and precise diagnosis, enabling improved clinical outcomes (Buczinski et al. 2010).

Reported clinical signs of cardiac affections in animals include peripheral oedema, brisket oedema, dyspnoea, tachycardia, tachypnoea, distended or pulsatile jugular vein, long standing and engorged scleral vessels. Congestive heart failure (CHF) refers to the heart's inability to eject and receive blood; in bovines, it is characterized by respiratory distress on light exertion, weakness, and presence of effusion and oedema as a result of increased hydrostatic pressure and fluid retention (Constable et al. 2017). It is indicative of other advanced heart diseases like chronic valvular heart disease, dilated cardiomyopathy (DCM) or hypertrophic cardiomyopathy. The most common disease of the myocardium is DCM which is primarily a myocardial disorder (Nart et al. 2004).

Pericarditis is the inflammation of pericardium with accumulation of fluid or exudate between the visceral and parietal pericardium (Reef and McGuirk 2009). Pericarditis is not common but can be effusive, fibrinous, and constrictive. Traumatic pericarditis is studied extensively but other causes of pericarditis are rarely studied and mainly

Address for correspondence:

Dr. Tarun Kumar, Assistant Professor
Department of Veterinary Clinical Complex
College of Veterinary science
Lala Lajpat Rai University of Veterinary and Animal Sciences
Hisar, 125004, Haryana, India

E-mail: drtarun17984@yahoo.co.in
<https://actavet.vfu.cz/>

categorised under idiopathic pericarditis (Constable et al. 2017). Significant information gap exists in the prevalence of cardiac diseases in bovines with definite diagnosis.

A 2016 large-scale retrospective observational study found an increasing incidence of right sided congestive heart failure (RHF) in feedlot cattle at elevations $\leq 1,282$ m in the midwestern United States and Canada (Neary et al. 2016). The authors also pointed out that hypoventilation and respiratory diseases, in addition to hypobaric conditions, can be responsible for pulmonary alveolar hypoxia ultimately leading to heart failure. Bovine cardiac diseases especially in case of buffaloes have received little clinical attention as the early diagnosis is not done frequently, leading to progression of the clinical condition with grave prognosis. Although high altitude and traumatic causes have been very well studied in cattle (Neary et al. 2016; Sasikala et al. 2018; Moxley et al. 2019), very scarce literature is available over the different cardiac diseases of non-traumatic origin with definite diagnosis. Since no systematic clinical studies have been performed on water buffaloes focusing on non-traumatic cardiac diseases, it seems important to study such animals so that a better therapeutic, preventive and prognostic approach may be suggested. So, the present study was designed with the first objective to characterize different non traumatic cardiac affections in buffaloes with the help of 2-D and M-mode echocardiograms. The second objective of the study was to determine a possible correlation between haemato-biochemical estimates, cardiac biomarker findings, and disease in the studied population.

Materials and Methods

The present study was carried out to characterize different cardiac affections of non-traumatic origin in buffaloes reported at a veterinary referral hospital in India over a period of 8 months. Out of a total of 1,768 female buffaloes screened, 57 animals were suspected for cardiac diseases on the basis of clinical signs and symptoms. Six healthy buffaloes kept at a university farm were included in the study as a healthy control.

Ethical approval

The procedure and use of animals was approved by the Institutional Animal Ethics Committee, vide no. VCC/IAEC/2625-2647.

Clinical examination

Complete clinical examination included recording of rectal temperature, heart rate, respiration rate, coughing status, auscultation of lungs and heart for abnormal sound, ruminal motility, mucous membrane colour, brisket oedema, nasal discharge, respiratory distress, muffling of heart sound, jugular pulsation and distension, open mouth breathing and exercise intolerance.

Radiography

Thoracic and reticulo-diaphragmatic radiography was performed in all suspected clinical cases in a standing position using a ceiling-mount X-ray tube (Siemens X-ray machine, Goa, India) using 90 kV and 160 mA/s for reticulo-diaphragmatic area and 80 kV and 60 mA/s for thoracic area. Images were processed and interpreted through the Computed Radiography System (Konica Minolta PVT, LTD, Mumbai, India).

Echocardiography

Echocardiography in buffaloes was performed as described in literature (Braun et al. 2001) using the Nemio-XG® portable ultrasound machine (Toshiba, Otawara-Shi, Japan). The area over the heart (3rd-7th intercostal space) caudal to the elbow on both the right and left hemithorax was clipped and surgical spirit was used to clean the skin. The left and right legs were stretched using rope alternatively from the front to expose the cardiac window. Ultrasound gel was applied before examination. Various 2-D and M mode images (cursor placed on 2-D image for measurement of left ventricle indices) were recorded. Different views like right parasternal long axis four chambered view and left parasternal caudal long axis four chambered view were recorded and analysed. Indicators measured using M-mode echocardiography during the four chambered view included the left ventricular posterior wall thickness during systole (LVPWs), left ventricular posterior wall thickness during diastole (LVPWd), left ventricular internal diameter during systole (LVIDs), left ventricular internal diameter during diastole (LVIDd), interventricular septum thickness during systole (IVSs), interventricular septum thickness during diastole (IVSd), fractional shortening of the left ventricle (FS%), ejection fraction of the left ventricle (EF%), left ventricle end diastolic volume (LVEDV), left ventricle end systolic volume (LVESV) and ejection time (ET). The stroke volume (SV) and cardiac output (CO) were also calculated from LV echocardiographic measurements to determine overall cardiac performance. Sonograms obtained in similar view were also evaluated

for any abnormal changes around the heart such as the presence of pericardial effusion or its thickening with fibrin clots, thickening of the valves, dilation of the heart cavities, systolic dysfunction, and an abnormal myocardial echogenicity. All the images along with M-mode measurements were analysed offline for individual cases and categorization of clinical cases was done in comparison to healthy control.

Pericardial fluid collection and examination

Ultrasound guided pericardiocentesis was performed with the help of sterile 4-inch-long 14-gauge spinal needle. Depth of fluid in the cavity was determined while performing ultrasound and the needle was inserted under local anaesthesia at the 4th intercostal space. Pericardiocentesis was performed only in cases where the animal's owner provided informed consent; animals whose owners declined the procedure were excluded from undergoing pericardiocentesis. Pericardial fluid examination includes potential of hydrogen (pH), specific gravity, total protein, albumin, glucose, lactate dehydrogenase (LDH), creatinine and cholesterol estimation. Total cell count in fluid was calculated as per literature with some modification (Caroline et al. 2017) i.e., the volume of one large corner square: $1\text{ mm} \times 1\text{ mm} \times 0.1\text{ mm} = 0.1\text{ mm}^3$; the volume of four large corner squares: $4 \times 0.1\text{ mm}^3 = 0.4\text{ mm}^3$. Let 'N' be the total number of cells in four WBC squares i.e. in 0.4 mm^3 of undiluted fluid; then total number of cells in 1 mm^3 of undiluted fluid will be, $N \div 0.4 = N \times 2.5$. Field staining of fluid smear was done to evaluate any presence of inflammatory cells, bacteria, and red blood cells.

Bacterial isolation was done following guidelines mentioned in literature (Quinn et al. 2011). Fluid sample was streaked on nutrient agar, 5% sheep blood agar and MacConkey's lactose agar (SRL, SIASCO reagents, Mumbai, India) simultaneously. Confirmation was done by colony characteristics and biochemical testing.

Haematobiochemical examination

Jugular venous blood (5 ml) was collected from affected and healthy control animals with 2 ml into K₂EDTA vacutainers for haemogram and 3 ml into silica vacutainers, centrifuged (1,000 g, 5 min), serum aliquoted and stored at $-20\text{ }^{\circ}\text{C}$ for biochemical, electrolyte, and cardiac biomarker analyses. Haematological indicators were estimated using automated haemato-analyser (MS4s, Melet Schloesing Laboratories, Rham, France). The erythrocytic indices measured were haemoglobin in g/dl and packed cell volume (PCV) in %. The leukocytic indices measured were the total leukocyte count (TLC) in $10^3/\text{mm}^3$, differential leukocyte count including neutrophils in %, lymphocytes in %, monocytes in % and eosinophils in %. Biochemical indicators, namely, total protein (g/dl), albumin (g/dl), LDH, aspartate aminotransferase (AST), glucose, blood urea nitrogen (BUN) (mg/dl), creatinine (mg/dl), triglycerides (TGA), alkaline phosphatase (ALP) and cholesterol were estimated using automated Random-Access Clinical Chemistry Analyzer (EM Destiny 180, Erba Diagnostics Mannheim GmbH, Germany) and electrolytes, i.e. sodium, potassium and chloride, were estimated by fully automated Easylyte[®] expand analyser (HD Consortium, India Ltd., New Delhi, India).

Cardiac biomarker estimation

Cardiac biomarker bovine cardiac troponin I (cTn-I) was estimated using Sandwich ELISA kit (Shanghai Korain Biotech Co., Ltd, China) and absorbance at 450 nm was taken with Synergy-2 Multi Detection Micro Plate Reader (Thermo Fisher Scientific, Shanghai, China). Serum samples stored at $-20\text{ }^{\circ}\text{C}$ were used for bovine cTn-I estimation within two weeks of collection and were compared with healthy control group kept in a healthy herd of university farm. A standard curve was constructed by plotting the average optical density for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and a best fit curve was drawn through the points on the graph. The best fit line was determined by regression analysis.

Statistical analysis

One-way ANOVA was used for comparing diseased and healthy animals with the help of statistical software package (SPSS 23). The results are presented as mean \pm standard error of the mean at the significance level of $P < 0.05$.

Results

Occurrence of non-traumatic cardiac affections in water buffaloes

Out of the total of 1,768 cases of female buffaloes reported over period of eight months, 57 animals were suspected for cardiac affections, of which 26 were confirmed non-traumatic cardiac cases. Age wise distribution revealed that the maximum number of cases were within the age group of 3–6 years (46.15%), followed by 6–9 years (42.30%), 1–3 years (3.8%), 9–12 years (3.8%) and over 12 years (3.8%).

Echocardiographic findings in water buffaloes affected with cardiac diseases

Cardiac cases were characterized under idiopathic CHF, CHF due to DCM, and constrictive pericarditis based on 2-D and M-mode echocardiographic findings. The cardiac

indices measured in clinical cases were compared with those determined in control animals kept under standard conditions at the university farm. Different mean values of M-mode indicators are shown in Table 1 and derived functional indices are presented in Table 2.

Table 1. Echocardiographic indicators of water buffaloes affected with different cardiac diseases (values are expressed as mean \pm standard error of the mean).

Indicator	Idiopathic CHF (n = 12)	CHF due to DCM (n = 8)	Constrictive pericarditis (n = 2)	Control (n = 6)
LVPWd (cm)	2.60 \pm 0.28	2.19 \pm 0.25	2.71 \pm 0.21	1.94 \pm 0.28
LVPWs (cm)	3.75 \pm 0.33	2.91 \pm 0.21	3.65 \pm 0.34	2.96 \pm 0.35
LVIDd (cm)*	4.81 \pm 0.44 ^a	6.59 \pm 0.33 ^b	4.23 \pm 0.69 ^a	5.46 \pm 0.38 ^a
LVIDs (cm)**	2.13 \pm 0.40 ^a	4.56 \pm 0.23 ^b	1.90 \pm 0.32 ^a	3.02 \pm 0.30 ^a
IVSd (cm)	2.44 \pm 0.19	2.37 \pm 0.35	2.25 \pm 0.71	1.85 \pm 0.28
IVSs (cm)	2.82 \pm 0.15	2.73 \pm 0.25	2.50 \pm 0.73	2.50 \pm 0.36

CHF - congestive heart failure, DCM - dilated cardiomyopathy, LVPWd - left ventricular posterior wall thickness during diastole, LVPWs - left ventricular posterior wall thickness during systole, LVIDd - left ventricular internal diameter during diastole, LVIDs - left ventricular internal diameter during systole, IVSd - interventricular septal thickness during diastole, IVSs - interventricular septal thickness during systole.

* Denotes significant differences between groups at $P < 0.05$; ** denotes significant differences between groups at $P < 0.01$; a,b different superscripts within rows represent significant differences

Table 2. Functional indices derived from echocardiographic indicators of buffaloes affected with different cardiac diseases (values are expressed as mean \pm standard error of the mean).

Indicator	Idiopathic CHF (n = 12)	CHF due to DCM (n = 8)	Constrictive pericarditis (n = 2)	Control (n = 6)
FS (%)	56.50 \pm 5.77 ^a	29.92 \pm 4.32 ^b	77.00 \pm 12.00 ^a	45.04 \pm 2.74 ^a
LVEDV (ml)	122.86 \pm 25.73 ^a	228.21 \pm 26.17 ^b	83.40 \pm 31.20 ^a	174.54 \pm 31.80 ^{ab}
LVESV* (ml)	25.98 \pm 13.48 ^a	98.01 \pm 12.28 ^b	2.20 \pm 1.60 ^a	31.9 \pm 8.90 ^a
EF (%)**	82.16 \pm 5.62 ^a	54.11 \pm 6.87 ^b	96.50 \pm 3.50 ^a	82.77 \pm 2.61 ^a
SV (ml)	96.85 \pm 16.95	130.2 \pm 23.86	81.4 \pm 32.8	142.61 \pm 25.03
CO (l/min)	8.29 \pm 1.44	10.18 \pm 1.67	7.70 \pm 3.03	9.09 \pm 1.91
ET (ms)**	697.57 \pm 36.15 ^a	773.37 \pm 59.33 ^a	684.0 \pm 44.0 ^a	1,050.66 \pm 125.55 ^b

CHF - congestive heart failure, DCM - dilated cardiomyopathy, FS - fraction shortening, LVEDV - left ventricle end diastolic volume, LVESV - left ventricle end systolic volume, EF - ejection fraction, SV - stroke volume, CO - cardiac output, ET - ejection time.

* Denotes significant differences between groups at $P < 0.05$; ** denotes significant differences between groups at $P < 0.01$; ^{a,b} different superscripts within rows represent significant differences

Idiopathic congestive heart failure in water buffaloes

Out of 26 confirmed cardiac cases, a total of 16 clinical cases were characterized as of idiopathic CHF based on 2-D and M-mode echocardiographic findings, which are listed below.

2-D echocardiographic findings

Cases of idiopathic CHF were diagnosed with the presence of pleural and pericardial effusion along with hyperechoic pericardium. In the present study, 2-D echocardiography demonstrated anechoic pleural effusion with varying quantity, with or without free floating echogenic fibrins in all 16 cases (100%), followed by pericardial effusion in 15 (93.7%)

cases when assessed on 2-D echo. Thickened pericardium was seen in 12 (75%) animals whereas fibrinous changes around the heart were observed in 11 (68.7%) cases (Plate I, Fig. 1). Pericardium was hyperechoic and easily demarcated by its increased thickness. Hyperechoic pericardium and epicardium were also separated by varying quantity of hypoechoic to mixed echogenic exudates in 15 (93.7%) cases. Floating echogenic fibrin shreds were often observed along with pericardial effusion, and sometimes these shreds bridged epicardium and pericardium. Reduction in the size of cardiac chambers was well appreciated in 13 (81.2%) animals.

M-mode examination

Out of sixteen animals, M-mode parameters were not taken in four buffaloes due to the presence of an excessive amount of pericardial and pleural effusion causing heart displacement from its normal position, making it difficult to take different indicators although thickened pericardium and mixed echogenic exudates were clearly visible in these four cases.

With the help of M-mode left ventricular (LV) diameter, LV wall thickness and LV systolic function was evaluated. Increased FS% and EF% with increased internal diameter of LV during diastole is suggestive of increased preload and categorised as CHF. The mean values of LVPWd (2.60 ± 0.28), LVPWs (3.75 ± 0.33), FS% (56.5 ± 5.77) and EF% (82.16 ± 5.62) were numerically increased while mean values of LVIDd (4.81 ± 0.44), LVIDs (2.13 ± 0.40), LVEDV, LVESV, SV and CO (8.29 ± 1.44) were numerically decreased compared to normal values recorded from healthy buffaloes kept as control. Mean values of IVS thickness during diastole as well as systole were more or less similar to normal values. Mean values of ET (697.57 ± 36.15 ; $\alpha = 0.01$) were significantly decreased compared to normal control values.

Congestive heart failure due to dilated cardiomyopathy in water buffaloes

Out of the 26 affected buffaloes, eight buffaloes were found to be affected with CHF due to DCM based on both 2-D and M-mode echocardiographic findings.

2-D echocardiographic findings

On 2-D examination diagnosis of DCM was given on the basis of visible dilation of all the heart chambers. In this study, DCM affected cases revealed dilation of LVIDd and systole (Plate I, Fig. 2) and the heart appeared to be distended and enlarged. Pleural effusion with or without fibrinous changes was present in the thoracic cavity in 75% animals.

M-mode examination

Dilated cardiomyopathy was only diagnosed with the help of M-mode measurements, where decreased FS% and EF% with increased internal diameter of LV during both diastole and systole are suggestive of cardiac enlargement and impaired systolic function. The mean values of LVIDd (6.59 ± 0.33 ; $\alpha = 0.05$) and LVIDs (4.56 ± 0.23 ; $\alpha = 0.01$) were significantly increased compared to healthy animals. Mean values of FS (29.92 ± 4.32 ; $\alpha = 0.05$) and EF (54.11 ± 6.87 ; $\alpha = 0.01$) were significantly reduced while mean values of LVEDV were numerically increased but a significant increase was found in LVESV (98.01 ± 12.28 ; $\alpha = 0.01$) compared to control animals. Mean values of SV were reduced while mean values of CO (10.18 ± 1.67) were found to be slightly increased compared to healthy control animals.

Constrictive pericarditis in water buffaloes

Out of the 26 affected buffaloes, only two were affected with constrictive pericarditis.

2-D echocardiographic findings

On 2-D examination, diagnosis of constrictive pericarditis was given on the basis of thickened and bright pericardium with no pleural effusion. In the studied animals, 2-D findings suggested thickened pericardium in both (100%) cases, although no pleural effusion was observed (Plate II, Fig. 3). Fibrinous changes around the heart were present in all the clinically affected animals.

M-mode examination

Constriction of cardiac chambers can be represented by LV measurements during diastole and systole. In the present study mean values of LVIDd, LVIDs, LVEDV, LVESV, SV and CO were found to be decreased whereas mean values of LVPWd, LVPWs, IVSd, FS and EF were found to be increased compared to healthy animals. Significant reduction in LVIDs ($\alpha = 0.01$) was observed while a significant increase was observed in FS% (77.0 ± 12.0 ; $\alpha = 0.05$) and EF% (96.5 ± 3.5 ; $\alpha = 0.05$) (Plate II, Fig 4 and Plate III, Fig 5).

Radiographic examination

Radiographic findings were inconclusive and mainly include poor thoracic details and indistinguishable cardiophrenic silhouette in all the categories.

Clinical examination

Major clinical signs in animals affected with cardiac affection in the present study were jugular distension (81.25%), muffled heart sound (71.87%) and brisket oedema (68.75%) followed by exercise intolerance (65.27%), long standing (53.1%) and moderate dyspnoea (50%). The least observed sign was coughing (37.5%) and elbow abduction (9.3%).

Idiopathic CHF affected animals showed presence of peripheral oedema which was present only in the brisket region in 63.1% cases whereas oedema involving the brisket, neck, and ventral abdomen in 15.8%. Exercise intolerance was present in 63.2% animals whereas 52.6% animals were showing the behaviour of long standing. Scleral vessels were found to be injected in 42.1% clinically affected cases. The heart rate was significantly increased (86.87 ± 3.95).

Animals affected with CHF due to DCM showed a slight increase in the mean values of heart rate (81.87 ± 7.61), pulse rate (75.87 ± 6.64) and respiration rate (38.62 ± 5.75) compared to normal values. Jugular veins were markedly distended in 75% (6) animals followed by jugular pulsation in 62.5% (5) clinical cases. Scleral vessels were injected in only 37.5% (3).

Constrictive pericarditis affected animals did not show any ventral oedema, only brisket oedema was evident, and they were not hesitant in sitting and standing. The animals did not have an abducted elbow although they had a bilateral ventral distension of the abdomen indicative of ascites with loss of appetite.

Haematological and electrolyte findings

Detailed haematological and electrolyte alterations in all cardiac categories compared to healthy control are listed in Table 3. Neutrophilia with hypocalcaemia are the key haemato-electrolyte alterations in constrictive pericarditis affected animals.

Biochemical alterations in cardiac affections

Hypoproteinaemia was observed in all cases of DCM ($\alpha = 0.01$) and CHF ($\alpha = 0.01$) with a significant decrease in albumin contents in DCM ($\alpha = 0.01$), CHF ($\alpha = 0.01$) and constrictive pericarditis ($\alpha = 0.05$). Mean LDH was numerically increased in DCM and constrictive pericarditis but significantly increased in CHF ($\alpha = 0.05$). Mean AST values were numerically increased in DCM and CHF but significantly increased in constrictive pericarditis ($\alpha = 0.01$). Mean glucose values were numerically increased in DCM and CHF but significantly increased in constrictive pericarditis ($\alpha = 0.05$). Mean BUN values

were numerically increased in CHF but significantly increased in constrictive pericarditis ($\alpha = 0.01$) and DCM ($\alpha = 0.01$). Cholesterol was significantly decreased in DCM ($\alpha = 0.01$), CHF ($\alpha = 0.01$) and constrictive pericarditis ($\alpha = 0.05$). No significant changes were observed in creatinine, TGA, and ALP in any of cardiac categories (Table 4).

Cardiac biomarker (cardiac troponin-I) estimation

In all the cardiac categories of bovines, cTn-I was increased drastically compared to the healthy control group as shown in Fig. 6 (Plate III). Significant increase ($P < 0.01$) was observed in buffaloes affected with DCM (186 ± 33.38), idiopathic CHF (170.75 ± 15.73) and constrictive pericarditis (128.01 ± 4.14).

Table 3. Haematological and electrolyte alterations in water buffaloes affected with different cardiac diseases (values are expressed as mean \pm standard error of the mean).

Indicator	Idiopathic CHF (n = 16)	CHF due to DCM (n = 8)	Constrictive pericarditis (n = 2)	Control (n = 6)
Hb (g/dl)	9.81 \pm 0.55	11.10 \pm 1.19	8.45 \pm 1.16	11.35 \pm 0.34
TEC $\times 10^6$ mm ³	5.91 \pm 0.41	6.12 \pm 0.24	6.23 \pm 2.15	5.58 \pm 0.28
TLC $\times 10^6$ mm ³ *	13.66 \pm 1.17 ^b	10.59 \pm 0.99 ^b	16.05 \pm 11.56 ^b	8.10 \pm 0.88 ^a
N (%)**	81.87 \pm 1.38 ^b	81.13 \pm 2.76 ^b	75.0 \pm 12.00 ^b	52.83 \pm 2.91 ^a
L (%)*	16.68 \pm 1.29 ^b	17.37 \pm 2.53 ^b	23.50 \pm 12.5 ^b	45.83 \pm 2.91 ^a
M (%)	1.37 \pm 0.23	1.12 \pm 0.12	1.50 \pm 0.50	1.33 \pm 0.21
PCV (%)	31.73 \pm 2.05	32.53 \pm 2.04	32.45 \pm 13.45	27.17 \pm 0.95
Ca (mg/dl)	8.68 \pm 0.34 ^b	8.63 \pm 0.52 ^b	8.10 \pm 0.01 ^b	10.57 \pm 0.56 ^a
Na ⁺ (mEq/l)	134.33 \pm 1.04	135.50 \pm 1.66	137.60 \pm 0.20	133.62 \pm 0.56
Cl ⁻ (mEq/l)	92.69 \pm 5.40	100.56 \pm 2.74	100.85 \pm 0.85	98.80 \pm 1.40
K ⁺ (mEq/l)	4.76 \pm 0.54	4.73 \pm 0.41	3.99 \pm 0.69	4.03 \pm 0.12

DCM - dilated cardiomyopathy, CHF - congestive heart failure, Hb - haemoglobin, TEC - total eosinophil count, TLC - total leukocyte count, N - neutrophils, L - lymphocytes, M - monocytes, PCV - packed cell volume

* Denotes significant differences between groups at $P < 0.05$; ** denotes significant differences between groups at $P < 0.01$; ^{a,b} different superscripts within rows represent significant differences

Table 4. Serum biochemical alterations in buffaloes affected with different cardiac diseases (values are expressed as mean \pm standard error of the mean).

Indicator	CHF due to DCM (n = 8)	Idiopathic CHF (n = 16)	Constrictive pericarditis (n = 2)	Control (n = 6)
Total protein (g/dl)**	6.87 \pm 0.43 ^b	6.82 \pm 0.38 ^b	7.38 \pm 1.16 ^a	9.73 \pm 0.75 ^a
Albumin (g/dl)**	1.94 \pm 0.11 ^b	1.72 \pm 0.07 ^b	1.95 \pm 0.27 ^b	3.48 \pm 0.22 ^a
LDH (IU)	2,554.75 \pm 684.26 ^b	2,490.43 \pm 355.78 ^b	1,756.00 \pm 584.00 ^b	1,031.50 \pm 122.67 ^a
AST (IU)	535.08 \pm 290.157 ^b	299.35 \pm 81.67 ^b	178.75 \pm 13.95 ^b	110.37 \pm 8.72 ^a
Glucose (mg/dl)	66.70 \pm 9.45 ^a	117.11 \pm 17.87 ^b	100.10 \pm 14.20 ^b	74.67 \pm 3.57 ^a
BUN (mg/dl)**	47.00 \pm 3.87 ^b	86.67 \pm 16.90 ^b	65.30 \pm 11.20 ^b	31.28 \pm 2.60 ^a
Creatinine (mg/dl)	2.28 \pm 0.56	2.45 \pm 0.44	2.15 \pm 0.02	1.71 \pm 0.11
TGA (mg/dl)	14.77 \pm 1.73	14.27 \pm 2.00	13.61 \pm 3.88	11.53 \pm 1.02
ALP (IU/l)	108.87 \pm 29.68	132.93 \pm 24.80	117.00 \pm 79.00	153.00 \pm 8.74
Cholesterol (mg/dl)	46.75 \pm 2.59 ^b	58.68 \pm 5.38 ^b	45.00 \pm 8.00 ^b	129.83 \pm 18.0 ^a

DCM - dilated cardiomyopathy, CHF - congestive heart failure, LDH - lactate dehydrogenase, AST - aspartate aminotransferase, BUN - blood urea nitrogen, TGA - triglycerides, ALP - alkaline phosphatase.

* Denotes significant differences between groups at $P < 0.05$; ** denotes significant differences between groups at $P < 0.01$; ^{a,b} different superscripts within rows represent significant differences

Bacterial isolation from pericardial fluid

Ultrasound guided pericardiocentesis was attempted in eight clinically affected cases having pericardial effusion. Out of eight pericardial fluid samples, 87.5% (7) were positive for bacterial culture. *Streptococcus* spp. (45.4%, 5) was the most common bacteria isolated in pericardial fluid followed by *E. coli* spp. (36.4%, 4), *Staphylococcus* spp. (9.1%, 1), and *Corynebacterium* spp. (9.1%, 1). Bacterial isolates were present alone as well as multiple bacterial species were also observed as listed in Table 5.

Table 5. Different isolates from pericardial fluid in selected cases.

Case No.	Nature of sample	Bacteria isolated
1	Pericardial fluid	<i>Streptococcus</i> spp. & <i>E. coli</i> spp.
2	Pericardial fluid	<i>E. coli</i> spp.
3	Pericardial fluid	<i>Corynebacterium</i> spp.
4	Pericardial fluid	<i>Streptococcus</i> spp. & <i>Staphylococcus</i> spp.
5	Pericardial fluid	<i>Streptococcus</i> spp. & <i>E. coli</i> spp.
6	Pericardial fluid	<i>Streptococcus</i> spp.
7	Pericardial fluid	<i>Streptococcus</i> spp. & <i>E. coli</i> spp.

Physical and biochemical examination of pericardial fluid revealed that pericardial fluid was whitish (62.5%) or whitish yellowish (37.5%) in colour, watery (25%) to milky consistency (75%) and foul smelling (100%) in nature. The pH and specific gravity of fluid were found to be 7.50 ± 0.18 and 1.0063 ± 0.0015 , respectively. Mean values of total protein were 2.49 ± 0.53 , albumin was 1.07 ± 0.25 , glucose was 15.89 ± 4.15 , creatinine was 0.54 ± 0.13 , LDH was $2,242.00 \pm 530.33$ and cholesterol was found to be 30.37 ± 7.26 .

On the basis of Light's criteria, out of 8 samples, 87.5% (7) samples were exudates and 12.5% (1) transudate. Mean TLC in pericardial fluid was $51.65 \pm 2.08 \times 10^5/\text{ml}$. Field staining revealed the presence of inflammatory cells in 87.5%, necrotic cell debris in 87.5%, fibrin in 75% along with bacteria in 62.5% of samples.

Discussion

Non-traumatic cardiac affections in water buffaloes

In the present study, occurrence of non-traumatic cardiac affections in buffaloes was found to be 1.47%. The highest occurrence (27.98%) of various pathological conditions of cardiovascular system was recorded in cattle in Rajasthan, India (Kumawat et al. 2020). The overall mean age of buffalo affected with cardiac issues was found to be 6.21 ± 0.56 years which means that buffaloes at their peak lactation are more prone to cardiac affections compared to young and old animals. Similar findings were also previously reported but most of them were for cattle (Puri 2016).

Aetiology of pericarditis may be infectious or idiopathic. In early stages of inflammation of the pericardium, hyperaemia occurs followed by deposition of fibrinous exudates (Constable et al. 2017). In constrictive pericarditis, the parietal pericardium, visceral pericardium or both develop(s) fibrosis. Pericardial fluid is not a consistent finding but sometimes is present in constrictive pericarditis (MacDonald 2017). In the present study, fibrosis of pericarditis was also evident along with presence of a small amount of pericardial effusion in only one case. Echogenicity of pericardial fluid totally depends upon its composition, such as the protein content, necrotic debris of inflammatory cells and bacterial cells, fibrins, mucopolysaccharides and nucleoproteins. In the present study,

evaluation with 2-D echocardiography showed presence of anechoic to fibrinous pleural fluid in the thoracic cavity in cases of idiopathic CHF as well as in CHF due to DCM that may be attributed to passive venous congestion developed due to cardiac insufficiency. Pulmonary oedema develops due to venous congestion with the presence of fluid around the septal vessels and in the alveolar spaces (Braun et al. 2008; Constable et al. 2017). As effusion develops, the inflamed surfaces are separated and accumulated fluid compresses the atria and right ventricle, preventing their complete filling and thus further aggravating the clinical conditions (Buczinski et al. 2010). Quantification of ventricular dilation and measuring systolic dysfunction are considered to be the diagnostic test of choice for diagnosing DCM in most species, so in the present study the final diagnosis of all the cardiac categories including DCM was done only after analysing different measurements and not only on the basis of visual appearance on 2-D echocardiography.

Echocardiographic findings in water buffaloes affected with cardiac diseases

The results of the present study showed that there was a numerical reduction in the mean values of LVIDs, LVIDd, LVEDV, LVESV, CO, SV and an increase in the mean values of FS% and EF% in idiopathic CHF compared to healthy control animals, whereas in constrictive pericarditis affected buffaloes a significant decrease was observed in LVIDs and significant increases were observed in systolic function performance indices such as FS% and EF%. Reduction in cardiac indices in presence of excessive pericardial fluid is suggestive of mechanical compression of the left ventricle chambers in case of CHF, which has also been reported previously in cattle (Braun et al. 2001). In cases of constrictive pericarditis, only a small amount of pericardial fluid was detected in one case, while no fluid was observed in the other. This suggests that cardiac chamber constriction resulted primarily from fibrotic thickening of the pericardium rather than fluid accumulation. In animals with CHF, mechanical compression and impaired diastolic filling led to incomplete ventricular and atrial filling, resulting in reduced left ventricular internal diameter (LVID) and consequent decreases in associated cardiac indices (Boon 2011).

Congestive heart failure due to DCM affected buffaloes showed a significant increase in the mean values of LVIDd, LVIDs, LVESV compared to healthy buffaloes suggesting poor LV systolic and diastolic functions. All the DCM affected animals also showed a significant decrease in mean values of EF% and FS%. The three primary factors affecting systolic performance indices are preload, afterload, and contractility. Thus, EF and FS may be low because of poor preload, increased afterload or decreased contractility (Hussain 2014). Changes in these cardiac indices in the DCM affected animals in the present study are due to an increase in the afterload which cause an increase in LVID and a decrease in the SV, resulting in a decrease in FS% and EF%. As per literature to date, no study on DCM in buffaloes has documented cases diagnosed on the basis of M-mode echocardiographic indicators.

Radiographic examination

Consistent findings on radiography in buffalo were loss of thoracic and cardiac details which might be due to accumulation of fluid in the pleural space (Hussain 2014) giving the whole radiography image a ground glass appearance. In all the radiographs of the present study, the heart was not well demarcated and visualized for interpretation. All the studied cases were negative for foreign body in reticulo-diaphragmatic and thoracic radiographs. Buffalo of this region are usually heavy-weight, with more than 500 kg body weight, and presence of fluid makes it difficult to take proper radiograph with interpretation of chambers size. Poor differentiation of thoracic contents on radiography with loss of contour of the diaphragm and poor cardiac silhouette was also reported previously

(Misk and Semieka 2001). Radiography is thus not a diagnostic imaging tool of choice for cardiac diseases in adult buffalo.

Clinical examination

The most common clinical signs in all 26 affected buffaloes were jugular distension, muffled heart sound, brisket oedema, exercise intolerance, long standing and respiratory distress. In case of idiopathic CHF, accumulated pericardial effusion leads to retrograde increased venous pressure and development of clinical signs like jugular distension, injected sclera vessels, peripheral subcutaneous oedema (De Fox et al. 1999). Muffling of the heart sound in the present study may be because of increased distance between the heart and stethoscope possibly due to a shift in the heart's normal position as well as due to increased pericardial fluid, fibrous tissue, changes in the pleural space or increased subcutaneous fat (De Moraes and Schwarz 2005). In early stages of cardiac disease, circulatory equilibrium may be maintained but cardiac reserve is reduced and the animal is not able to cope with circulatory emergencies. At this stage, the animal is relatively normal but unable to perform exercise or move efficiently for grazing and watering, therefore exercise intolerance develops (Constable et al. 2017). The mean heart rate was increased in all cardiac categories in the present study which is a compensatory mechanism of the heart to decreased CO. So, it was difficult to categorise clinical cases into different cardiac affections based on clinical signs as most of the signs are present in all diseased animals.

Haemato-biochemical examination

In the present study, neutrophilic leukocytosis observed in all categories of cardiac affections as a necessary reaction by the body to heal or ward off an invading microorganism or foreign substance (Aiello and Moses 2016). Increased PCV in the present study indicative of polycythaemia which might be due to haemoconcentration either due to dehydration or systemic hypoxia. Significant hypocalcaemia with hypoalbuminaemia was observed in all cardiac categories similarly reported in cattle (Shah 2010). Significant hypoproteinaemia and hypoalbuminaemia may be attributed to the hepatic congestion and loss of hepatic functions (Buczinski et al. 2010). Increase in LDH has been reported to occur after an injury to the liver, skeletal muscle, cardiac muscle, and kidney (Meyer and Harvey 2004). Hence, the significantly higher value of LDH in cardiac cases of buffalo indicates severity of cardiac muscle damage, in addition to liver involvement in the disease. As CO is decreased in CHF and constrictive pericarditis renal ischaemia or reduced renal perfusion occur, subsequent pre-renal azotaemia causes increased BUN and creatinine values in serum, as recorded in all the cardiac categories of buffaloes in the present study. Similar findings were also reported earlier (Tharwat 2011; Puri 2016). The increased activities of AST in all the cardiac categories could be attributed to the hepatic congestion (Braun 2009) reflecting hepatocellular damage, secondary to passive congestion and centrilobular hypoxia associated with the heart failure.

Significant decrease in cholesterol values was observed in all cardiac categories of buffaloes. Total cholesterol levels in serum decrease in severe liver dysfunction or in cases of hypoalbuminaemia (Sharma et al. 2004). Since cholesterol is mostly lipophilic, it is transported through the blood, along with TGA, inside lipoprotein particles (HDL, LDL, VLDL, and chylomicrons). These lipoproteins are detected in the clinical setting to estimate the amount of cholesterol in the blood. So, it has been hypothesized that due to severe liver dysfunction there is a decrease in synthesis of these carrier protein and therefore its value in blood is decreased. So, it can be stated that multiple organs may get involved in cases affected with cardiac diseases leading to poor prognosis of the patient.

Cardiac biomarker examination

The results of this study indicate that estimation of cTnI is a useful diagnostic marker for the confirmation of cardiac affections in buffaloes, but cannot differentiate different cardiac myopathies or affections. The elevated levels of bovine cTnI only reflected myocardial damage and cardiac insufficiency in this study as was also stated by Gunes et al. (2008).

Bacterial isolation from pericardial fluid

Presence of bacterial isolates in the pericardial effusion indicates chronicity of disease, immunosuppression and continuous insult to the pericardium leading to infective pericardial effusion. Furthermore, as the present study was conducted at a university referral hospital, most cases were presented after a prolonged course of illness, which was evident from the extended duration of clinical signs observed in the affected animals. Pericardial fluid accumulates in the pericardial sac as a result of inflammation of the pericardium, increase in atrial or ventricular pressure or of idiopathic reason, which become effusive and serosanguineous as the disease progresses (Sharma et al. 2004). Exudative effusive pericarditis may happen because of chronicity of infection (Fishman et al. 2006). Hypoglycaemia in pericardial fluid may be a result of glucose utilization by bacterial cells. High LDH level indicated cardiac muscle damage.

In the end it was concluded that non traumatic cardiac affections in buffaloes are important clinical conditions and can be diagnosed and characterized with echocardiography whereas radiography did not show promising results. Cardiac biomarker cTn-I proved to be a sensitive diagnostic aid for overall cardiac affections but not able to differentiate between different categories, whereas increased LDH indicates cardiac as well as multiple organ involvement. Pericardial fluid having exudative nature with *Streptococcus* spp. and *E. coli* as major isolated bacteria was indicative of disease chronicity. Limitations of the study include a small number of control animals for comparison with diseased animals and no standard data available for measurements of different cardiac indices in water buffalo. Thus, results will be verified after obtaining normal data using a large number of control/healthy water buffaloes.

We were able to classify cardiac affections and DCM was reported for the first time in water buffaloes. Characterization of non-traumatic cardiac affections in farm animals especially high milk yielders will be an important step in further understanding different mechanisms of heart failure and cardiomyopathies.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors thank Dean Cows, Dean PGs, HOD Veterinary Medicine, HOD Veterinary Hospital, LUVAS-Hisar, and the HOD department of LPM.

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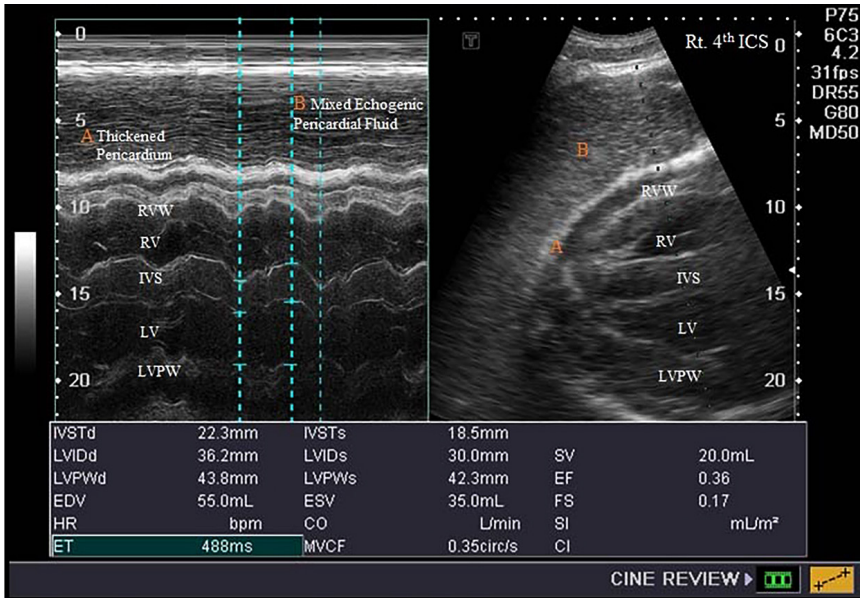


Fig. 1. Right parasternal long axis view of the heart at the 4th intercostal space (ICS) (M-mode) in idiopathic congestive heart failure-affected buffalo

RVW - right ventricle wall, RV - right ventricle, IVS - interventricular septa, LV - left ventricle, LVPW - left ventricular posterior wall

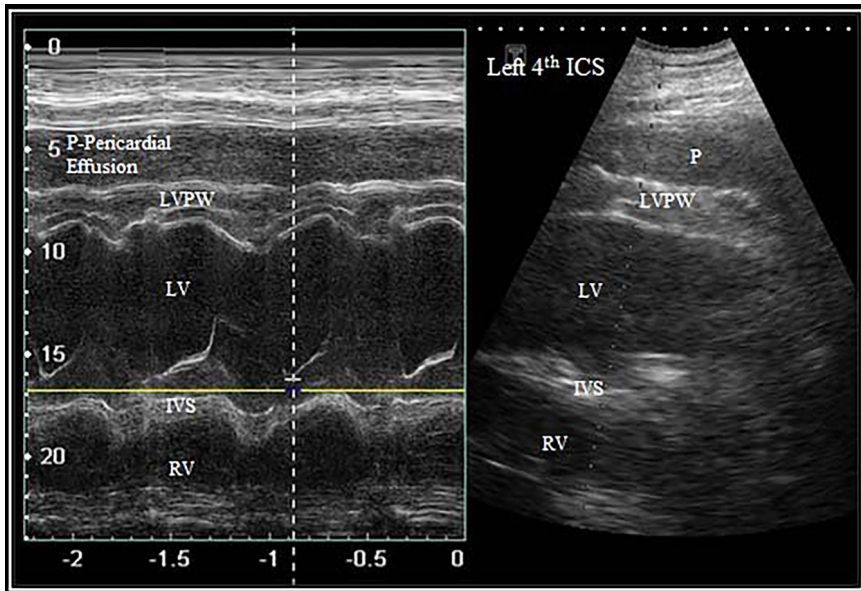


Fig. 2. M-mode examination of the heart (left 4th intercostal space, ICS) in congestive heart failure due to dilated cardiomyopathy-affected buffalo

LVPW - left ventricular posterior wall, LV - left ventricle, IVS - interventricular septa, RV - right ventricle

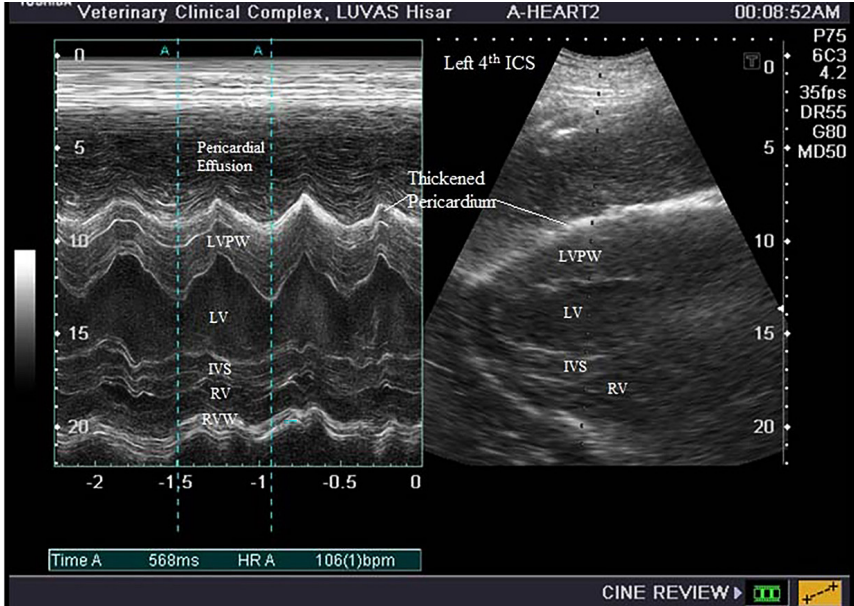


Fig. 3. M-mode examination of the heart (left 4th intercostal space, ICS) in constrictive pericarditis-affected buffalo LVPW - left ventricular posterior wall, LV - left ventricle, IVS - interventricular septa, RV - right ventricle, RVW - right ventricle wall

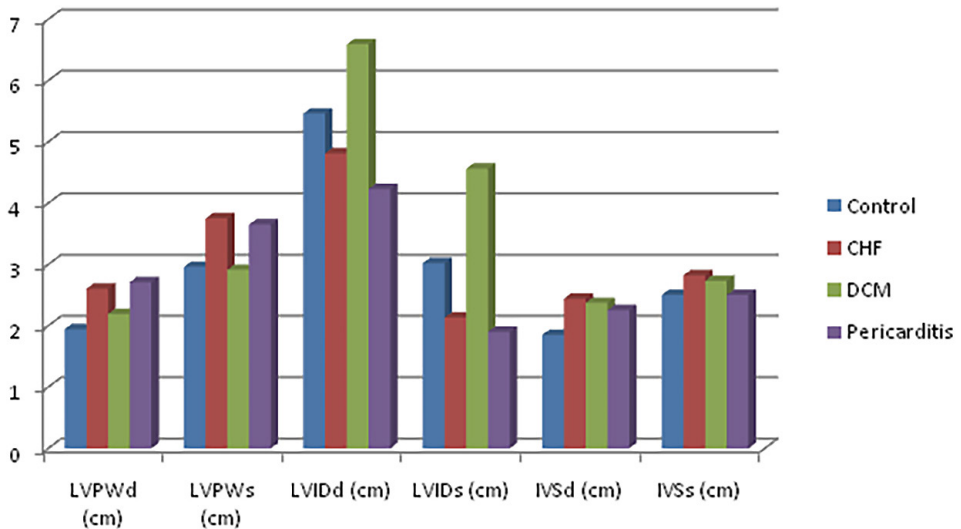


Fig. 4. Comparison of echocardiographic indicators in control and cardiac affected buffaloes CHF – congestive heart failure; DCM – dilated cardiomyopathy; LVPWd – left ventricular posterior wall thickness during diastole; LVPWs – left ventricular posterior wall thickness during systole; LVIDd – left ventricular internal diameter during diastole; LVIDs – left ventricular internal diameter during systole; IVSd – interventricular septum thickness during diastole; IVSs – interventricular septum thickness during systole

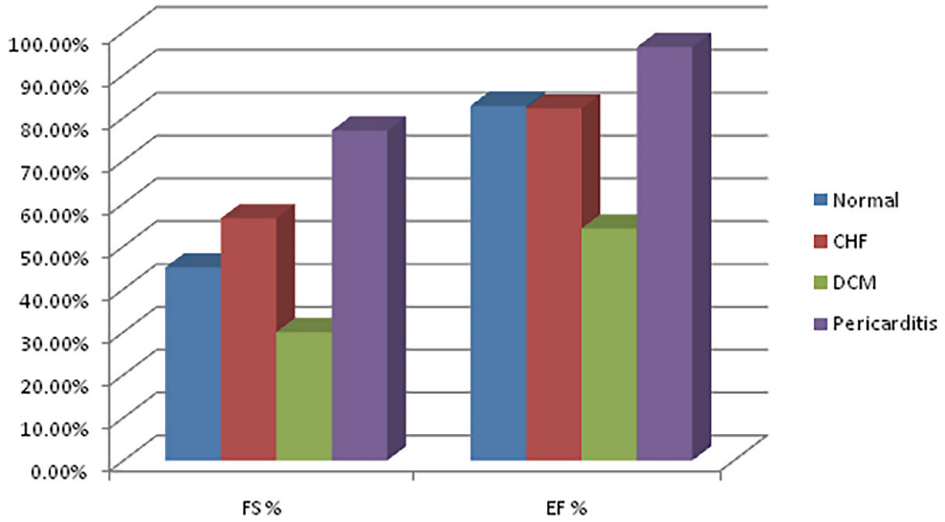


Fig. 5. Comparison of FS% and EF% in control and cardiac affected buffaloes
 CHF – congestive heart failure; DCM – dilated cardiomyopathy; FS – fractional shortening; EF – ejection fraction

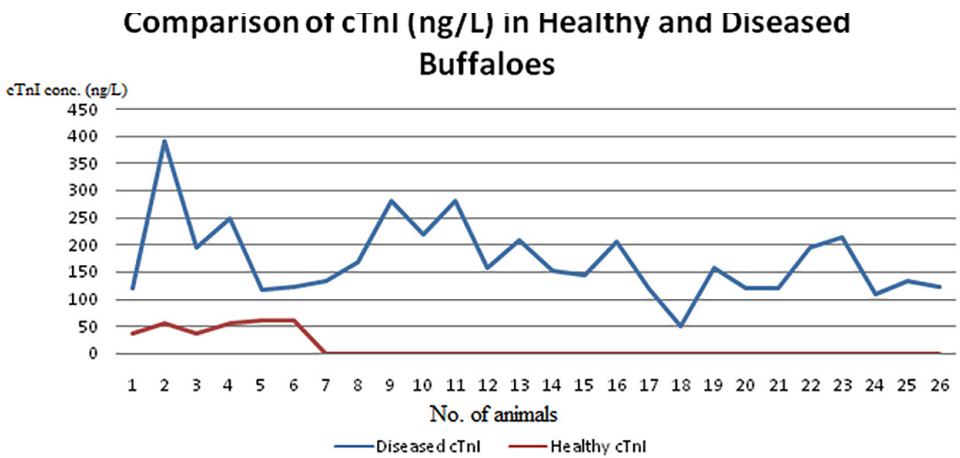


Fig. 6. Cardiac troponin (cTnI) analysis in control and cardiac affected buffaloes