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Research Article

ULTRASONOGRAPHIC FINDINGS AND CARDIAC TROPONIN ASSESSMENT IN TRAUMATIC RETICULO-PERICARDITIS AFFECTED COWS

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ABSTRACT

Foreign body syndrome in cattle is a major challenge for both farmers and veterinarians. This study was aimed to evaluate the ultrasonographic findings of the heart, reticulum, spleen, liver, portal vein, hepatic vein, gall bladder and caudal vena cava in bovine traumatic reticulo-pericarditis (TRP) affected cows. Among 230 cows screened for foreign body syndrome, at the Large Animal Medicine Referral Clinics, Veterinary College and Research Institute, Orathanadu, Thanjavur from July 2019 to June 2020, 41 cows were found to have TRP. Blood biochemistry revealed liver enzyme elevations mainly AST and LDH along with hypoproteinaemia. Creatinine kinase myocardial fraction level was elevated. Electrocardiography revealed lower amplitudes of R complex. Radiography showed ground glass appearance of thoracic cavity. Ultrasonographic examination revealed varying degree of pericardial effusion and reticular abnormalities, which included uneven wall surfaces, abscesses and reduced reticular contraction. Engorged portal vein and distended gallbladder were noticed. Cardiac Troponin (cTnI) was elevated (0.15 ± 0.03 ng/ml) significantly in comparison to that of healthy animals. Thoracic and abdominal ultrasonography helped in detecting cardiac and various intra-abdominal organ abnormalities associated with TRP and Cardiac Troponin assessments helped in confirmation of myocardial image in affected cows.

Keywords: Cattle,Traumatic Reticulo-Pericarditis (TRP), Ultrasound, Cardiac Troponin

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Foreign body syndrome in cattle is a major challenge for both farmers and veterinarians. In cows with traumatic reticulo-pericarditis, an early, precise diagnosis and proper therapeutic measuresas well as prognostication are necessary.For identifying the presence of foreign body in the thoracicor abdominal cavity, radiography is being used (Braun, 1993; Khalphallah *et al.*, 2015); however sometimes diagnostic yields are less with radiography. Ultrasonography remains an early and best approach for detecting pericarditis and/or reticulitis, as well as different pathological pericardial effusions and reticular adhesions in animals, wherein radiography cannot detect such changes (Braun 2009; Khalphallah *et al.*, 2015). Cardiac Troponin (cTn-I) was an excellent biomarker of cardiac injury in all mammals (O'Brien *et al.*, 1997). Kyung Chan Park (2017) stated that cTnI was the gold-standard marker for acute myocardial necrosis: the pathological hallmark of acute myocardial infarction (AMI). This study was aimed to evaluate the diagnostic utility of radiography, ultrasonography and cardiac biomarkers in the diagnosis of pericardial affections in cows.

MATERIALS AND METHODS

The study was conducted from July 2019 to June 2020, at the Large Animal Medicine Referral Clinics, Veterinary College and Research Institute, Orathanadu, Thanjavur. During this study, 230 dairy cows were evaluated for foreign body syndrome. 41 cows were found to had TRP based on the history, clinical signs and special examinations comprising of ECG, X-ray, ultrasonography and cTn-I evaluations.These 41 cases were selected initially based on their clinical signs like edema in submandibular area and brisket edema (Fig. 1a & b). All the 41 animals were subjected to detailed clinical examinations, ECG, X-ray and Ultrasound examinations. Electrocardiography was performed on animals in standing position employing base apex lead system placement (Rezakhani *et al.*, 2004 and Yogeshpriya *et al.*, 2019). Radiography was performed on standing animals using a right lateral approach with a focal film distance of 90-100 cm (using an 800 mA X-ray equipment with a setting of 90-100 kVp & 50-60 mAs) according to the Braun *et al.* (1993).

Ultrasonography was performed using Esaote My Lab one version with curvy linear probe of 2.5 to 5 mHz as per standard protocols of Braun *et al.* (2009) and Venkatesan *et al.* (2019). Ultrasonographic scanning was done for the heart and left lateral thoracic wall at 3rd to 5th intercostal space, reticulum (on left lateral thoracic wall at 6th to 8th intercostal space), spleen on the left side, right kidney (behind the last rib), liver (on right side at 9th to 12th intercostal space) and caudal vena cava (on right side at 11th

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intercoastal space). While right parasternal approaches were also used in this study, we also used the 3rd to 5th left intercostal space approach as it helped in obtaining clear images.

Two ml of blood was collected in EDTA and clot activator tubes for haematology, serum biochemistry and cardiac biomarker assessments. Haematology was done using auto analyzer (Vet Scan HM5, Abbott, UK) using manufacturer's recommended kits. The samples collected in clot activator vials were kept without disturbing at room temperature for 1 hr and centrifuged at 3000 rpm for 15 minutes, and the serum was separated and stored at -20°C until analysed. Blood Urea Nitrogen (BUN), Total Protein, Albumin, Glucose, Creatine Kinase -Myocardial band (CK-MB); Alkaline Phosphatase (ALT), Aspartate aminotransferase (AST); Lactate dehydrogenase (LDH), Calcium (Ca) and Phosphorus (P) levels were determined using an automated biochemical analyser with commercially available biochemistry test kits, as per manufacturers recommended protocols.

Cardiac Troponin was evaluated in all the 41 cows in serum samples using the Point-of-Care immunoassay i-STAT analyser (Abbott Healthcare Pvt. Ltd) (Venkatesan *et al.*, 2020) (Fig. 2a). Electrolytes (K, Na, Cl Total calcium and ionized Ca) and pH were assessed with a semi-automated Electrolyte analyser (All Care AC 9801, Allied Biotechnology India Pvt. Ltd.) (Yogeshpriya *et al.*, 2017).

The independent sample student 't' test was used for analysis of data by using SPSS software @ 25.0 version

RESULTS AND DISCUSSION

Foreign body syndrome remains a major economic and clinical challenge in cattle and farmers and veterinarians often find difficulty in decision making. In this study, out of 230 animals suspected and evaluated for foreign body syndrome, 41 cows were found to had TRP. Out of these 41 cows, 29 were crossbred Jersey, 8 were crossbred Holstein Friesian and 4 were non-descript. Among the 41 cows, 21 were milking, 9 were dry and 11 were in their last trimester of pregnancy. This highlights the economic challenges the poor farmers face when their cows are affected with TRP.

Physical examinations parameters included mild elevation of temperature (39.8±0.3°C), elevation of Heart rate (91±2 bpm) and presence of muffled heart sounds in the 25 affected cows (Table 1). Affected cows had anaemia with low Hb and low erythrocytes count, leukocytosis and neutrophilia. Blood biochemistry revealed liver enzyme elevations mainly in AST and LDH values along with hypoproteinaemia (Table 2). Creatinine kinase myocardial fraction level was found to be elevated. The cTnI fraction was elevated significantly ($p<0.05$) than the healthy animals (Table 2). No significant changes were observed on the electrolytes between the healthy animals and TRP affected cows. These findings were in accordance with the previous reports (Tharwat, 2011; Khalphallah *et al.*, 2015 and Venkatesan *et al.*, 2020).

Based on clinical presentation, the cows were initially diagnosed to have TRP and were subjected to detailed examinations. In the ECG, 'R' complex amplitude was reduced in animals which had traumatic pericarditis and with a substantial amount of fluid accumulations. Similar findings were reported earlier by Reef *et al.*, 2002 and Torki *et al.*, 2010). Tharwat (2011) reported of small QRS complexes and short T waves in the sixteen pericarditis affected cows. In the present study too, similar electrocardiographic changes were observed in the affected animals (Fig. 2b).

Radiographic assessment revealed that 21.95% of cows had the presence radio-opaque foreign body in thoracic cavity and 17.07% of the cows had radio-opaque foreign body of varying lengths and shapes in the reticulum. 60.97% of cows showed ground glass appearance of thoracic cavity on radiographic examination (Fig. 3a-3d). These radiographic findings of indistinguishable nature of cardiac silhouette and the ground glass appearances were in accordance with Misk *et al.*, 2001 who opined that radiography could provide good thoracic details in the earlystages of traumatic pericarditis, and poor differentiation of thoracic structures when the adhesions were progressed and severe. For these reasons ultrasonographic assessment are being used increasingly.

Ultrasonography of the heart revealed pericardial effusion of varying nature (Table 3); anechoic fluid was observed in 17 cows and was suggestive of serous form of pericarditis (Fig. 4a); homogenous effusions in pericardium with echoic particles were observed in 14 cows and were suggestive of septic pericarditis (Fig. 4c); anechoic fluid with extensive hyperechoic fibrin strands were observed in 10 cows and were suggestive for constrictive pericarditis (Fig. 4e). Similar findings were also reported in previous studies (Premkumar *et al.*, 2019; Khalphallah *et al.*, 2015).

Ultrasonography of Reticulum revealed of wall thickening in 9 cows and presence of abscess in 4 cows; uneven reticular surface was observed in 18 cows and presence of effusive fluids in-between the thoracic wall and reticular wall was observed in 10 cows (Fig. 5a, b.). Ultrasonography of Liver parenchyma revealed no major changes except for dilated caudal vena cava (2.7±0.12 cm) in 19 cows, distension of hepatic vein and portal veins in 22

Fig. 1. **(a)** Picture showing jowl and brisket edema in a Traumatic reticulopericarditis affected Crossbred Jersey Cow; **(b)** Picture showing jowl, brisket and ventral abdominal edema of Traumatic reticulopericarditis affected Crossbred Jersey Cow

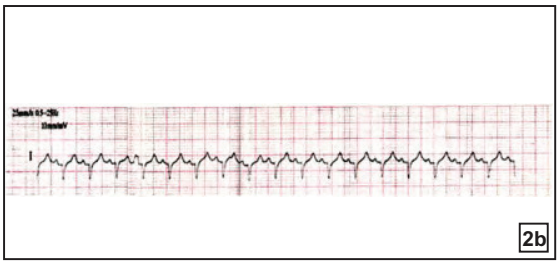
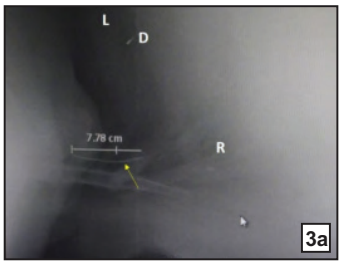
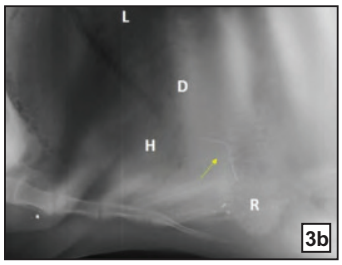
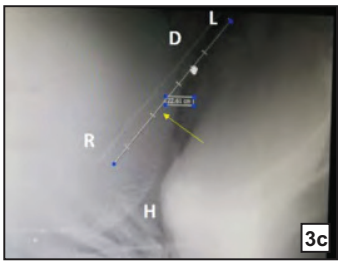


Fig. 2. **(a)** i-STAT analyser result shows Cardiac Troponin I (0.89 ng/ml) value in cow with TRP. **(b)** Electrocardiogphy Base apex showing decreased QRS complexes in TRP cow.







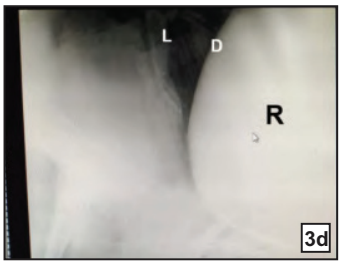
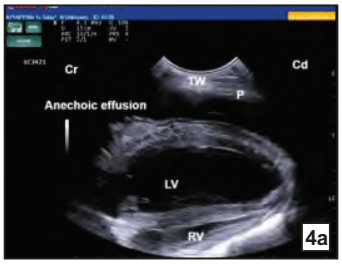


Fig. 3. **(a)** Left lateral thoraxic radiography 7.78 cm radio opaque piercing foreign body visualized at 3rd to 4th rib left lateral thoraxic radiography (Arrow). L- Lung, D – Diaphragm, R – Reticulum. **(b)** Left lateral radiography showing radio-opaque piercing foregin body present in reiculum (Arrow). L- Lung, H- Heart, D – Diaphragm, R – Reticulum. **(c)** Left lateral radiography cavity showing radio-opaque piercing foregin body lengh around 22.1 cm passing through reticulum (Arrow). L- Lung, H- Heart, D – Diaphragm, R – Reticulum. **(d)** Left lateral thoraxic radiography of showing distingusable cardiac silhoutte due to pericardial effucion.L- Lung, D – Diaphragm, R – Reticulum.



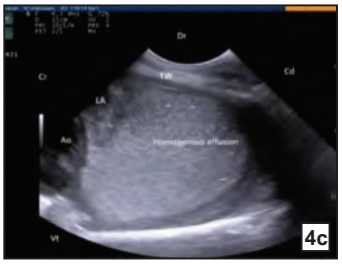


Fig. 4. **(a)** B-Mode Ultrasonography of heart at left side 4th ICS, showing anechoic pericardial effusion in traumatic reticlulopericarditis affected cow.TW- Thoracic wall, P- pericardium, LV- Left Ventricle, RV- Right Ventricle, Cr -Cranial, Cd- Caudal; **(b)** Clear transudate with frothy pericardial effusion (1.5 L) of the same cow was drained with ultrasound guidance; **(c)** B-Mode Ultrasonography of heart at left side 4th ICS, showing Heterogenous pericardial effusion in traumatic reticlulopericarditis affected cow. Where, TW- Thoraxic wall, LA- Left Atrium, Ao- Aorta, Cr-Cranial, Cd- Caudal; **(d)** Foul smelling light yellowish pericardial effusion (1.0 L) the same cow was drained with ultrasound guidance.

Fig. 4. **(e)** B-Mode Ultrasonography of heart at right side 4th ICS, showing hyperechoic fibrin with anechoic pockets of pericardial effusion in traumatic reticlulopericarditis affected cow.TW- Thoraxic wall, P- pericardium, RV- Right Ventricle, RA- Right Atrium, Cr -Cranial, Cd- Caudal; **(f)** Foul smelling heamorrhagic pericardial effusion (0.5 L) drained from the same cow, was drained by ultrasound guidance

Fig. 5. **(a)** B-Mode Ultrasonography of reticulum at left thoraxic cavity 7th ICS, showing uneven wall thickness with presence of anechoic fluid in TRP cow.TW- Thoraxic wall, D- Diaphragm, R- Reicular wall. **(b)** B-Mode Ultrasonography of reticulum at left 7th ICS, showing uneven reticular wall with presence of anechoic fluid and floating of spleen in TRP cow.

cows. These could be indicative of early stages of congestion. Gall bladder was visualized with distension along with wall thickening (2.5±0.62 mm) in 23 cows (Fig. 6c, d).

Echocardiography had good sensitivity when compared to necropsy findings in cattle with cardiac disease (Buczinski *et al.*, 2010) and in the current study, even cows without clinical signs of pericarditis showed pathological changes ultrasonographically. M-mode ultrasonography the four-chamber view of cardiac was obtained from the left parasternal long-axis view when the

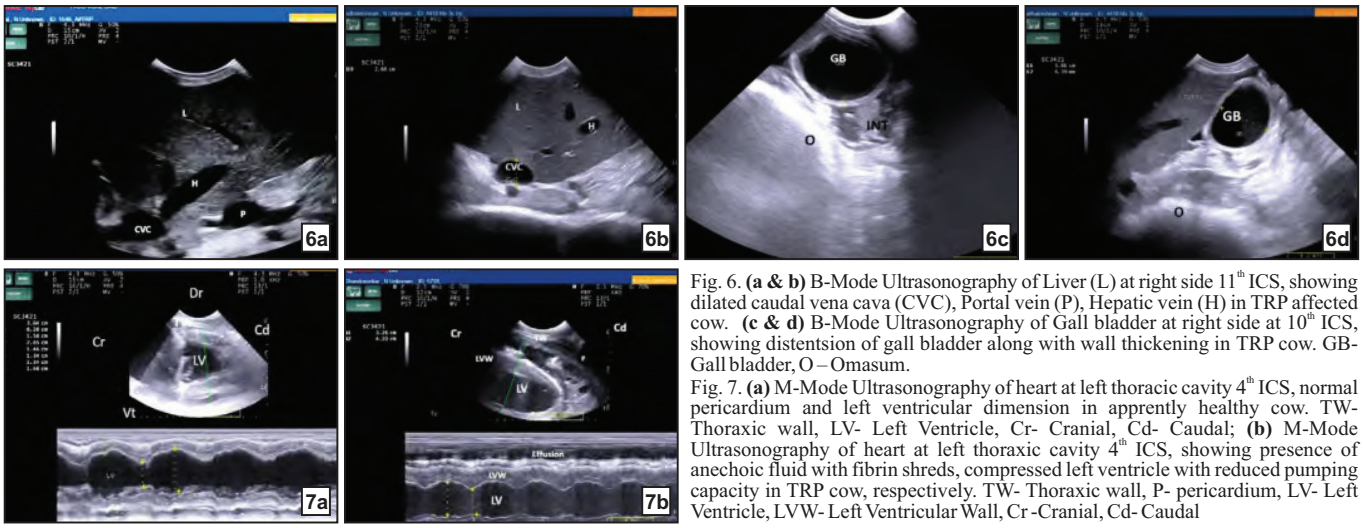


Fig. 6. **(a & b)** B-Mode Ultrasonography of Liver (L) at right side 11th ICS, showing dilated caudal vena cava (CVC), Portal vein (P), Hepatic vein (H) in TRP affected cow. **(c & d)** B-Mode Ultrasonography of Gall bladder at right side at 10th ICS, showing distentsion of gall bladder along with wall thickening in TRP cow. GB- Gall bladder, O – Omasum.

Fig. 7. **(a)** M-Mode Ultrasonography of heart at left thoracic cavity 4th ICS, normal pericardium and left ventricular dimension in apprently healthy cow. TW- Thoraxic wall, LV- Left Ventricle, Cr- Cranial, Cd- Caudal; **(b)** M-Mode Ultrasonography of heart at left thoraxic cavity 4th ICS, showing presence of anechoic fluid with fibrin shreds, compressed left ventricle with reduced pumping capacity in TRP cow, respectively. TW- Thoraxic wall, P- pericardium, LV- Left Ventricle, LVW- Left Ventricular Wall, Cr -Cranial, Cd- Caudal

Table 1. Physical parameters in cows with traumatic reticulo-pericarditis

Parameter	Control animals (n= 19) (Mean ± SE)	TRP cows (n= 41)(Mean ± SE)
Temperature (^{0}C)	38.3 ± 0.1	39.8 ± 0.3
Heart rate (bpm)	56 ± 3	91 ± 2
Heart intensity	Normal (Louder)	Low (Muffled; 25 cows)
Jowl / Brisket edema	No	Present
Positive venous stasis	No	Present
Lymphnode	Optimal size	Normal to slightlyenlarged
Jugular pulsation	No	Present
Rumen consistency	Normal	Doughy to impacted
Rumen motility (per 5 min)	3 ± 0.02	1 ± 0.1

Table 2. Haematology, cardiac and related biomarkers of cows with traumatic reticulo-pericarditis

Parameter	Control cows (n=19)	TRP cows (n=41)
BUN mg /dl	20.32 ± 0.93[a]	41.72 ± 5.74[b]
Creatinine (mg /dl)	2.46 ± 1.14	0.90± 0.07
Glucose (mg/dl)	44.76 ± 2.87[a]	43.30 ± 4.6[b]
Total protein (g/dl)	5.91 ± 0.45[a]	5.57 ±0.25[b]
Albumin (g /dl)	2.88 ± 0.23[a]	2.88 ± 0.34[b]
ALT (U/L)	29.18 ± 3.30	29.18 ± 4.8
AST (U/L)	72.80 ± 6.40[a]	122.14 ± 6.67[b]
CK-MB (U/L)	57.68 ± 12.80[a]	202.34 ± 18.35[b]
LDH (U/L)	948.52±164.94	1650.26 ± 140.05
Calcium (mg/dl)	7.28±0.4	9.94 ± 0.59
Phosphorus (mg/dl)	5.34±0.2	6.47 ± 0.20
Magnesium (mg/dl)	2.36±0.12	2.47 ± 0.21
cTnI (ng/ml)	0.022 ± 0.006[a]	0.15 ± 0.03[b]
Hb (g/dl)	8.66 ± 0.31[a]	6.03 ± 0.29[b]
PCV (%)	23.33 ± 0.95	23.33 ± 1.38
RBC (mil/cmm)	5.64 ± 0.29[a]	5.64 ± 0.42[b]
WBC (/cmm)	5890.99 ± 349.93[a]	8389.28 ± 620.81[b]
Neutrophils (%)	43.18±1.57	52.12 ± 1.84
Lymphocytes (%)	38.59±1.75	41.40 ± 1.71
Monocytes (%)	3.89±1.06	3.08 ± 0.27
Basophils (%)	1.75±0.38	1.52± 0.20
Eosinophils (%)	0.83±0.35	1.78 ± 0.30
Potassium (mmo/L)	4.044 ± 0.212	3.74 ± 0.26
Sodium (mmo/L)	149.46 ± 7.77	150.16 ± 4.36
Chloride (mmo/L)	124.37 ± 5.10	93.05 ± 2.74
iCa (mmo/L)	0.95 ± 0.035	1.27 ± 0.77
pH	7.89 ± 0.10[a]	7.65 ± 0.06[b]

within rows means with different letters (a, b) differ significantly ($p < 0.05$)

Table 3. Ultrasonographic findings of cows with TRP

S.No.	Studied Organs	Ultrasound findings
1.	Pericardia effusions	Anechoic fluid (n=17 cows) Homogenous effusions (n=14 cows) Anechoic fluid with hyperechoic fibrin strands (10 cows)
2.	Reticulum	Wall thickening (n=9 cows) Abscess (n=4 cows) Uneven reticular surface (n=18 cows) Effusive fluids in-between the thoracic wall and reticular wall (n=10 cows)
3.	Spleen	Normographic study (n=41 cows)
4.	Liver Pharenchyma	Normographic study (n=41 cows)
5.	Caudal vena cava	Dilated (n=19 cows)
6.	Hepatic vein and portal veins	Distension (n=22 cows)
7.	Gall Bladder	Distension along with wall thickening (n=23 cows)
8.	Right Kidney	Normographic study (n=41 cows)

Table 4. Echocardiographic measurements and calculated cardiac indices in normal and traumatic reticulo-pericarditis cows (p<0.01)

Parameters (Unit)	Control cows (n=6) Mean ±SE	TRP Cows (n=6) Mean ±SE	t-value	p
LVWTd (mm)	24.7 ± 0.99	36.41 ± 0.95*	8.473	0.000
LVWTs (mm)	34.41 ± 1.78	25.68 ± 1.87	3.354	0.070
LVDs (mm)	33.26 ± 1.60	24.3 ± 0.55*	5.296	0.000
LVDd (mm)	68.51 ± 2.36	46.03 ± 1.0*	8.756	0.000
IVST (mm)	23.46 ± 0.99	23.51 ± 0.48	0.45	0.965
RVD (mm)	30.75 ± 1.83	22.9 ± 0.69*	1.520	0.003
RVWTs (mm)	24.22 ± 1.27	18.6 ± 0.49*	3.988	0.002
RVWTd (mm)	15.28 ± 1.54	13.4 ± 0.22	4.096	0.256
FS %	51.34 ± 2.25	47.08 ± 1.65	1.204	0.159

*Statistical Significance (p<0.01)

probe was placed at the 4th ICS or the 5th ICS from the left side Brudzinski (2009) (Fig. 7a, b). From our study echocardiographic indices LVWTd, LVDs, LVDd, RVD and RVWTs were of high statistical significance at (p<0.01) Table 4. LVDs, LVDd values were reduced when compared to control and our findings were similar to the earlier findings (Yamaga and Too, 1986) whereas IVST value did not very much.

Gunes and coworkers (2008) concluded that qualitative cTn kits were valuable in determining the degree of heart damage due to TRP. Early quantification of cTn-I would be of help in prognostication of cows with traumatic pericarditis. Previous reports (Venkatesan *et al.*, 2020) observed that elevation in cTnI (0.10 ± 0.03 ng l ml) levels was found in cows with TRP affected cows while that of healthy cows was 0.027 ± 0.016 ng / ml. Based on the clinical signs, radiographic and ultrasonographic findings, all the 41 cows were confirmed for TRP affections. This was further confirmed by elevated cTnI in all 41 cases. 23 cases were drained using ultrasound guided pericardiocentesis, while the rest of the animal owners were not willing for interventional procedures. Among the drained 23 cows, 11 were available for follow up to 1 monthly only; these owners also failed to report for further follow-up. With such poor owner compliance, prognosis could not be monitored over a longer period.

Prognosis was attempted based on the ultrasonography guided aspiration and nature of the aspirated fluid; those with purulent aspirate and sepsis were given few month of prognosis and all of them died.

CONCLUSION

Out of 230 cases suspected and examined for foreign body, 41 had TRP. Radiographic assessments revealed that 21.95% of cows had radio-opaque foreign body in thoracic cavity and 17.07% had radio-opaque foreign body in reticulum. In all 41 cases ultrasound exams confirmed the TRP and it was further confirmed by elevated cTnI in all the 41 cases. For better clinical decisions and to help farmers plan their course of actions in handling affected cows, cTnI shall be made part of the evaluation for all cases with TRP suspicions.

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