

Ultrasonography, Cardiac Biomarkers and Biochemical Analysis as a Diagnostic Tool in Traumatic Reticulitis and its Sequelae in Egyptian Buffaloes

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Abstract

This study was performed for early diagnosis of traumatic reticulitis (TR), traumatic reticuloperitonitis (TRP) and traumatic pericarditis (TP) in buffaloes based on clinical, ultrasonography and biochemical analysis. Fifty-eight buffaloes were admitted to the Veterinary Teaching Hospital at Zagazig University, Egypt. The investigated buffaloes were divided into 4 groups, Group I was the control, group II suffered from TR, group III suffered from TRP, and group IV suffered from TP. The most prominent clinical signs of diseased cases were anorexia, sudden, and severe reduction of milk yield, abnormal ruminal movement varied from ruminal atony and ruminal stasis and recurrent tympany. In addition, buffaloes with TP showed edema at the dewlap, distension of jugular veins and abnormal heart sound (muffled and tinkling sound). Ultrasonographically, peritoneum and pericardium appeared as either mild or massive anechoic accumulations of fluid containing fibrin thread corrugation of the reticular wall was varied from moderate to severe. Deposits of fibrinous tissue interspersed with fluid pockets were seen between the reticulum, dorsal ruminal sac and diaphragm. Serum troponin I (cTnI) and creatine kinase myocardial band (CK-MB) were significantly higher in the TP group compared with control, TR and TRP groups. Moreover, IL-6, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were significantly higher in the TRP and TP group compared with control and TR groups. Therefore, it was concluded that cardiac biomarkers and ultrasonography is an excellent tool for early prediction and diagnosis of TRP and TP in buffaloes. moreover, TP in buffaloes had more significant changes in biochemistry than the TRP buffaloes.

KEYWORDS

Buffaloes, Cardiac biomarker, Traumatic reticuloperitonitis, Traumatic pericarditis, Ultrasonography.

INTRODUCTION

Traumatic reticuloperitonitis (TRP) remains one of the most important internal disorders of cattle (Misk, 2015). As cattle are not very selective and cannot distinguish metallic objects in food. These materials can stick to the reticulum mucosa due to their anatomical conformation in a honeycomb shape without causing damage, however reticular physiological contractions, pressure of the fetus in advanced gestation and uterine contractions at the time of delivery, promote penetration of the reticular wall (Macedo *et al.*, 2021).

The small distance between the reticulum and the pericardium facilitates its perforation by a contaminated foreign body and the presence of a mixed bacterial infection of the reticulum causes severe local inflammation, with accumulation of serous or fibrinous inflammatory products, which can subsequently trigger toxemia and heart failure (Macedo *et al.*, 2021)

Ghanem (2010) stated that animals with TRP had anorexia, arched back, and a severe decrease in milk production, were reluctant to rise or move and exhibited anxiousness. Abduction of the elbow joints was also observed, indicating cranial abdominal pain, in addition to repeated chronic tympany. The TP cows had muffled heart sounds, jugular distension, and pulsation and edema of the brisket and submandibular regions.

In the veterinary field, TP is one of the most important cardiac diseases among bovines, it cause economic losses due to the sudden and sharp decrease of milk production, treatment costs, and finally losses of the animal as usually is associated with progressive disturbances in heart function (Attia, 2016).

The importance of this disease is not only due to its higher prevalence among other digestive disorders, but also due to the difficulty in early prediction and difficulty in evaluation of its sequelae by physical examination. Therefore, additional diagnostic techniques like ultrasonography and biochemical analysis are often helpful (Abdelaal *et al.*, 2009).

Cardiac biomarkers as troponin I (cTnI) and creatine kinase myocardial band (CK-MB) are used to detect cardiac dysfunctions, in addition it was reported that the serum activities of some enzymes, such as AST were elevated during myocardial damage (Ayvazoglu *et al.*, 2023). Beside, at the site(s) of inflammation or infection tissue release cytokines, including interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)- α that induce local and systemic reactions, typically pyrexia, hormonal variations, and muscle protein depletion and stimulate the hepatic release of APPs (El-Ashker *et al.*, 2013).

Therefore, the present study aimed to early diagnosis of TR and its most common complication, monitoring of the most common ultrasonographic and biochemical changes in TR, TRP

and TP.

Sampling

MATERIALS AND METHODS

Ethical approval

All procedures used in the present study were approved by the Scientific Research Ethics Committee on animal research, Faculty of Veterinary Medicine, Suez Canal University, Egypt.

Study period and location

The study was carried out at Veterinary Teaching Hospital at Zagazig University, Egypt, during January to June 2023.

Animal and study design

A total of 58 buffaloes were admitted to the clinic with a history of anorexia and decrease of milk production, these buffalo were classified according to clinical and ultrasonographic examination into:

Group I: including 10 apparently clinically healthy that were kept as control group.

Group II: including 10 case suffering from TR.

Group III: including 22 case suffering from TRP.

Group IV: including 16 cases suffering from TP.

Clinical examination

All animals were subjected to clinical examination according to the method of Jackson *et al.* (2002).

Ultrasonographic examination

The reticulum, rumen, liver, lungs and heart were examined with a 3.5- and 5-MHz convex transducer (Pie Medical 240 Parus, Maastricht, Netherlands). The hair was clipped on the ventral abdomen and on both sides of the thorax. The reticulum and surrounding tissues were examined as described by Braun and Götz (1994). The heart was examined as described by Braun *et al.* (2001). After the application of transmission gel, the animals were scanned beginning at the ventral abdomen and extending forward to the intercostal spaces on both sides of the thorax. Each intercostal space was examined dorsoventrally.

A total of 58 blood samples were collected as follows 10 samples from control group, 10 from group II, 22 from group III and 16 from group IV. About 5 ml of blood was collected from all investigated buffaloes by jugular vein puncture. Blood serum samples were harvested into plain tube in which it was allowed to clot for 30 - 60 min and then centrifuged at 3000 rpm for 10 min, and then kept frozen at -20°C until the further analysis (Kaneko *et al.*, 2008).

Biochemical analysis

Serum total proteins, albumin, and liver enzymes AST and ALT were measured calorimetrically using Commercial kits which were provided by Biomerieux, Egypt, Meanwhile, globulin concentration was calculated by subtracting albumin from total proteins and A/G ratio was calculated by dividing the albumin value over globulin value in addition, IL-6 was measured calorimetrically using Commercial kits (Kamiya Biomedical Company, USA). Also, the activities of cTnI and CK-MB were measured calorimetrically with commercial kits (Card-I-kit Combo Test; Aboa Tech).

Statistical Analysis

The obtained data of the present study were analyzed statistically using one way analysis of variances (ANOVA) for all tested groups according to Snedecor and Cochran (1967). Means separations were done by Duncan's Multiple Range test according to Duncan (1955). The present data were analyzed using (SPSS, 20) for windows. Data were presented as mean \pm standard error (SE). The results were considered significant at probability level of 0.05 ($P \leq 0.05$).

RESULTS

Clinical finding

The history and clinical findings of the TR, TRP and TP cases were showed in Table 1. The investigated animals revealed, anorexia, sharp decline in milk yield, recurrent tympany with markedly ruminal atony and ruminal stasis compared to ruminal movements in the clinically healthy buffaloes, increase in heart and respiratory rate, Muffled sounds and Tinkling heart sound, brisket edema and jugular pulsation was observed in TP.

Table 1. General clinical findings of TR, TRP and TP in buffaloes.

Parameters	Groups	Control (n=10)	TR (n=10)	TRP (n=22)	TP (n=16)
Heart rate(bpm)		62.3 \pm 1.5 ^d	67.0 \pm 1.5 ^b	76.33 \pm 0.88 ^a	96.67 \pm 1.6 ^c
Heart sound		Normal	Normal	Normal	Muffled sounds (n=11), Tinkling (n=5)
Respiratory rate (breath/min)		12.0 \pm 0.58 ^c	13.0 \pm 0.58 ^b	15.67 \pm 0.88 ^a	42.33 \pm 1.2 ^{dc}
Rectal temperature (c ^o)		38.6 \pm 0.08 ^a	38.96 \pm 0.03 ^a	38.7 \pm 0.39 ^a	38.57 \pm 0.69 ^a
Appetite		Normal	Variable degree of anorexia	Inappetence (n=18), Anorexia(n=4)	Inappetence (n=10), Anorexia(n=6)
Change in milk production		Absent	Slight decrease	Sharp decline	Sharp decline
Ruminal motility		Normal 3/2 min.	Hypermotility (n= 7), Hypomotile (n=3)	Hypomotile (n=8), Ruminal stasis (n=14)	Hypomotile (n=4), Ruminal stasis (n=12)
Recurrent tympany		Absent	Present (n=7), Absent (n=3)	Present (n=22)	Present (n=15), Absent (n=1)
Brisket oedema		Absent	Absent	Absent	Observed in long standing cases (n=14)
Jugular vein (distension and pulsation)		Absent	Absent	Absent	Distension with true pulsation (n=16)

Means carrying different superscripts in the same row are significantly different at ($P \leq 0.05$) or highly significantly different at ($P < 0.01$).

Ultrasonographic examinations

In case of traumatic reticulitis, reticulum appeared as a hyper-echoic halfmoon shape with inflammatory deposits on its serosal surface, Reticular motility slow with two contractions / 3min as shown in Fig. 1. Meanwhile, Acute diffuse peritonitis extended from just behind the xiphoid cartilage to the pelvic region in the ventral abdomen and characterized by extensive echogenic strands and hypoechoic peritoneal fluid in the ventral abdomen between xiphoid and pelvic region and reticulum appear as halfmoon shape with inflammatory deposits on its serosal surface with one contraction / 3min as shown in Fig. 2.

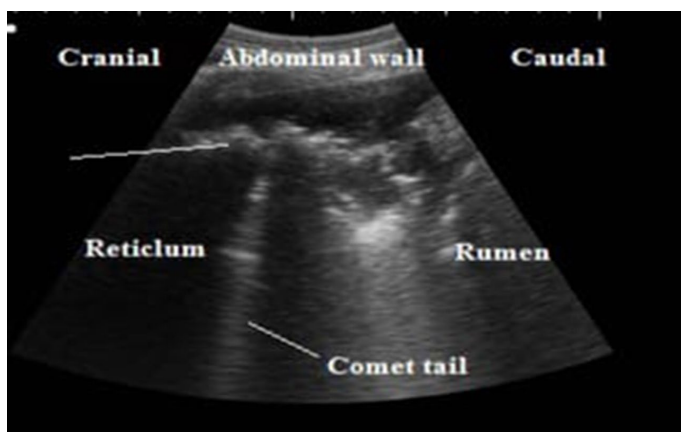


Fig. 1. Sonogram of a buffalo (3.5 MHz) with reticulitis. The ultra sonographic image shows echogenic corrugated half-moon shape of reticular wall and comet tail artifact as indicator for presence of foreign body.

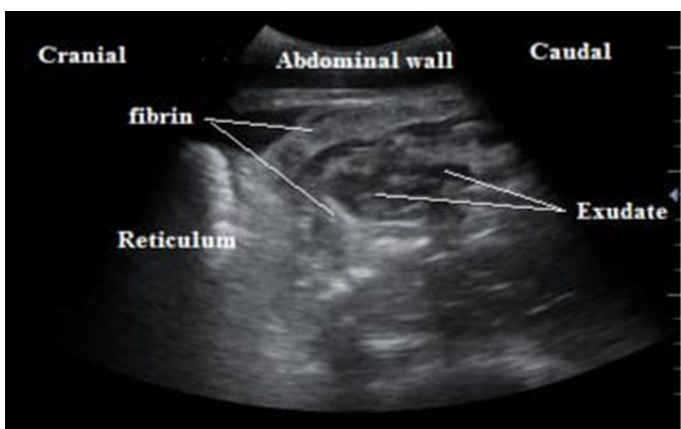


Fig. 2. Sonogram of a buffalo (3.5 MHz) with peritonitis. The ultrasonographic image shows echogenic fibrinous strands between reticulum and abdominal wall. Notice the corrugated appearance of the reticular wall.

All TP cases were extended from the 3rd and 4th intercostal spaces on both sides of the thorax and characterized ultrasonographically by deformity in the reticular wall and loss of both contour and half-moon shaped appearance. The reticulum was displaced from the diaphragm by a distance with either a fibrinous echogenic mass between the reticulum and the abdominal wall (caudoventral to the reticulum) or with echogenic fibrinous

deposits interspersed with hypoechoic pockets of fluids compared to those in healthy buffaloes (The reticulum-diaphragm distance = 0.5–1.4 cm). Reticular contractions were reduced to 0/3 min compared to those in healthy buffaloes (3/3 min).

Thoracic ultrasonographic findings included cardiomegaly, thickened cardiac wall with deformity in the characteristic cardiac shape. Accumulation of hypoechoic fluids (usually pus) was interspersed with echogenic deposits of fibrin within the pericardium as shown in Fig. 3.

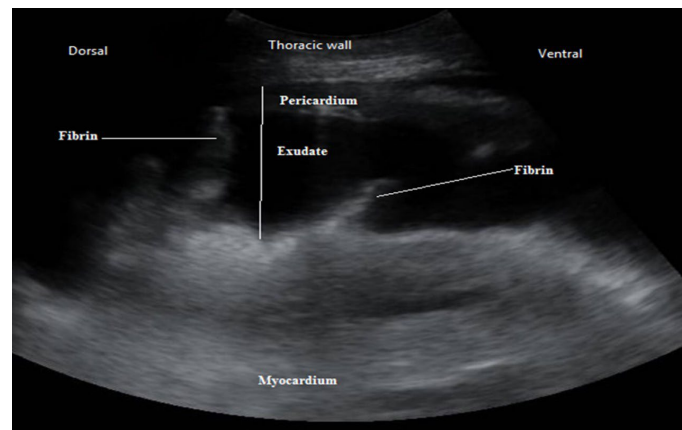


Fig. 3. Sonogram of the left thorax of a buffalo with pericarditis obtained from the 4th intercostal space, echogenic bands between the hypoechoic thick pericardium and the heart represent fibrin (3.5 MHz).

Serum biochemical analyses

A highly significant increase in the mean values of cTnI and CK-MB at $P \leq 0.05$ of buffalo with TP compared with the control group, TR and TRP. Meanwhile, a non-significant change in mean values of cTnI and CK-MB at $P \leq 0.05$ were recorded in buffalo with TR and TRP compared with control group as shown in Table 2.

Table 2. Cardiac biomarker in buffalo suffered from TR, TRP and TP.

Groups	Parameters	Troponin I (ng/mL)	Ck MB (U/L)
Control		0.03±0.005 ^b	131.4±0.21 ^b
TR		0.05±0.01 ^b	131.83±0.60 ^b
TRP		0.08±0.03 ^b	128.9±1.64 ^b
TP		1.52±0.31 ^a	190.66±5.60 ^a

Means carrying different superscripts in the same row are significantly different at ($P \leq 0.05$) or highly significantly different at ($P < 0.01$).

In proteinogram there are A significant decrease in the mean values of total protein at $P \leq 0.05$ were recorded in buffaloes with TRP and TP compared with TR and control group. Meanwhile, A significant decrease in the mean values of albumin at ($P \leq 0.05$) were recorded in buffaloes with TR, TRP and TP compared with control group. A significant increase in the mean values of globulin at $P \leq 0.05$ were recorded in buffaloes with TRP and TP compared with TR and control group.

Table 3. Inflammatory marker and proteinogram in buffalo suffered from TR, TRP and TP.

Groups	Parameters	IL-6 (pg/mL)	TP (g/dl)	Albumin (g/dl)	Globulin (g/dl)	AG ratio
Control		6.33±0.56 ^c	7.06±0.09 ^a	3.64±0.06 ^a	3.42±0.05 ^b	0.93±0.09 ^a
TR		17.6±1.24 ^b	6.76±0.43 ^a	3.24±0.07 ^b	3.52±0.49 ^b	1.01±0.16 ^a
TRP		31.03±0.03 ^a	5.92±0.19 ^b	1.4±0.04 ^c	4.52±0.15 ^a	0.31±0.005 ^b
TP		31.07±0.03 ^a	5.88±0.04 ^b	1.28±0.05 ^c	4.6±0.08 ^a	0.27±0.01 ^b

Means carrying different superscripts in the same row are significantly different at ($P \leq 0.05$) or highly significantly different at ($P < 0.01$).

A significant increase in the mean values of IL-6 at $P \leq 0.05$ of buffaloes with TR, TRP and TP compared with control group. Meanwhile, A significant increase in the mean values of IL-6 at $P \leq 0.05$ of buffaloes with TRP and TP compared with TR and control group as shown in Table 3.

A highly significant increase in the mean values of AST and ALT at $P \leq 0.05$ of buffalo with TRP and TP compared with TR and control group and a highly significant increase in the mean values of AST and ALT at $P \leq 0.05$ of buffalo with TP compared with TRP. Meanwhile, a non-significant change in mean values of AST and ALT at $P \leq 0.05$ were recorded in buffalo with TR compared with control group shown in Table 4.

Table 4. liver enzymes in buffalo suffered from TR, TRP and TP.

Groups	Parameters	AST (U/L)	ALT (U/L)
Control		123.33±0.88 ^c	26.33±0.88 ^c
TR		122.33±0.33 ^c	30.0±1.53 ^c
TRP		146.67±1.20 ^b	54.0±2.65 ^b
TP		166.66±1.20 ^a	62.66±1.20 ^a

Means carrying different superscripts in the same row are significantly different at ($P \leq 0.05$) or highly significantly different at ($P < 0.01$).

DISCUSSION

In the present study clinical cases of buffaloes with TR, TRP and TP were diagnosed and compared to healthy control depending on clinical, biochemical, and ultrasonographic changes. A non-significant change in rectal temperature was attributed to having been ill for several days and treatment before admission to the clinic, this result was in agreement with Braun *et al.* (2018).

The significant increase in the respiratory rate indicates respiratory distress associated with toxemia and septicemia caused by the foreign body penetration or due to cardiac insufficiency or direct involvement of the lungs (Ghanem, 2010).

Decreased or absent of rumen motility in the majority of TRP cases is explained by inhibition of the gastric centre in the medulla oblongata via the vagal nerve because of pain associated with a foreign body. However, Ruminal tympany, due to decreased eructation (Braun *et al.*, 2018).

In TP engorgement of jugular vein and brisket edema may be attributed to the pericardial effusion as the volume of fluid in the pericardial sac increased, increase the intracardiac pressure, constrain the cardiac output, impedes the venous return to the heart, increase the venous hydrostatic pressure causing the ventral edema (Braun, 2009).

Heart sound detected on TP varies depending mainly on the type of lesion and the severity of tachycardia depends primarily on the degree of compression of the heart by pericardial effusion, muffled heart sound heard may be attributed to pericardial effusion and fibrin changes, when the changes are mainly fibrin, the sounds are of a scratching in nature. Meanwhile, if the fluid was predominant, there are splashing or gurgling sounds, similar results previously described by Attia (2016).

In this study, ultrasonography, was successfully used for diagnosis of TR, TRP and TP in buffaloes, Reduction or completely absence of the reticular movement occurs due to adhesion with the abdominal wall. meanwhile, increase the distance between the pericardium and epicardium due to the presence of hypoechoic pericardial effusion "interspersed with echogenic deposits, representing fibrin strands (Flöck, 2004).

The reticulum was displaced from the diaphragm in the TP cases by 2.6-4.5 cm. This displacement was either due to the presence of a fibrinous echogenic mass between the reticulum and abdominal wall (caudoventral to the reticulum), or because of echogenic fibrinous deposits interspersed with hypoechoic pockets of fluids. The reduction in reticular movement in TP cases was attributed to the changes in the contour of the reticulum,

while in TRP cases depend on the severity of the inflammatory changes (Khalphallah *et al.*, 2017).

cTn proteins are usually present in blood either at very low concentrations or below the limit of detection, The significant increase of cTn-I and CK-MB concentration in TRP and TP affected group indicates myocardial cell damage and released into the circulation in pericarditis (Gunes *et al.*, 2008). The results of this study indicate that cTn-I are useful diagnostic aids for the confirmation of pericarditis in buffaloes especially those without pathognomonic signs "engorgement of jugular vein and brisket edema." Their elevated levels considered a reflection of myocardial damage and cardiac insufficiency (Attia, 2016).

TRP in buffalo are associated with cytokine production. Our results indicated that TRP in buffalo was associated with higher serum levels of IL-6 compared to the controls. The principal pathway leading to hepatic production of the APPs is the release of proinflammatory cytokines from macrophages at the site of inflammation. IL-6, TNF- α , and IL-1 β are key stimulators of hepatic APP production (El-Ashker *et al.*, 2013).

During inflammation, the production of protein in the liver is switched towards increased synthesis of globulin which is a positive acute phase proteins with a decrease in the synthesis of albumin which is a negative acute phase protein, increase in globulin concentration in the current work reflects the inflammatory reaction which occurred in the affected buffalo and a hypoproteinemia was observed in buffalo as in these cases the concentrations of the globulin fraction failed to compensate for the severe loss of albumin concentration. Hypoproteinemia was also reported in congestive heart failure and in severe acute peritonitis due to massive non-selective protein loss (Saleh *et al.*, 2008).

The significant increase of liver enzymatic activity of AST and ALT in the buffaloes with TRP and TP than in the controls is associated with impaired hepatic function that might be due to hepatic damage secondary to TRP. In addition, the significant increase AST and ALT in TP group than in TRP group, suggesting that more severe damage to the liver and muscles is associated with TP, These results are in agreement with those previously reported (Ghanem, 2010).

CONCLUSION

The results of the present study clear the importance of ultra-sonographic examination and biochemical analysis of buffaloes with TRP. More routine use of ultrasonography may aid in early diagnosis of TRP and detection of its sequelae which are difficult to be detected by clinical examination alone.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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