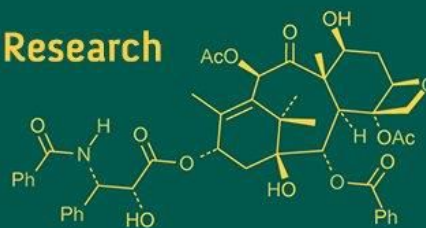


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Diagnostic utility of D-dimer and peritoneal fluid cytology in traumatic reticuloperitonitis in bovines

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Abstract

Traumatic reticuloperitonitis (TRP) is a prevalent and economically significant thoraco-abdominal disorder in bovines caused by ingestion of traumatic metallic foreign bodies leading to reticular perforation and septic peritonitis. Due to overlapping clinical features with other gastrointestinal disorders, diagnosis often remains challenging. This study aimed to assess the diagnostic utility of peritoneal fluid cytology and D-dimer concentration in bovines with confirmed TRP. Peritoneal fluid was collected from six affected and six healthy bovines, and evaluated for gross appearance, total protein, red and white blood cell counts, and D-dimer levels. Cytological smears stained with Giemsa were semi-quantitatively scored for polymorphonuclear cells (PMNC), mononuclear cells (MNC), fibrin, red blood cells (RBCs), and bacteria. TRP cases exhibited significantly elevated total protein ($p < 0.01$), RBCs, total leukocyte and neutrophil counts ($p < 0.05$), and D-dimer levels ($p < 0.05$) compared to controls. Cytology revealed increased PMNC scores and decreased MNC scores ($p = 0.0022$), with significantly higher fibrin deposition, bacterial presence, and RBC infiltration ($p < 0.05$). A strong positive correlation ($\rho = 0.926$, $p = 0.033$) between tube clot test and D-dimer concentration indicated active fibrinolysis concurrent with peritonitis. Based on cytological scoring, cases were stratified into mild, moderate, and advanced septic TRP stages, with findings ranging from sterile exudates to marked neutrophilic infiltration, fibrin clumps, and intra-/extracellular bacteria. This study demonstrates that the integration of peritoneal fluid cytology and D-dimer estimation provides a rapid, minimally invasive, and field-applicable diagnostic approach with high clinical relevance. The semi-quantitative cytological scoring system developed herein enhances the accuracy of TRP diagnosis and staging, particularly in field conditions where advanced diagnostics may be unavailable. Overall, this multimodal approach improves the early identification and management of septic peritonitis in bovines, reducing diagnostic ambiguity and improving prognosis.

Keywords: Bovines, D-dimer, fibrin, peritoneal fluid cytology, traumatic reticuloperitonitis, tube clot test

Introduction

Traumatic reticuloperitonitis (TRP) remains one of the most frequently encountered and economically significant thoraco-abdominal disorders in bovines, attributed largely to indiscriminate feeding behavior and increasing environmental exposure to metallic foreign bodies. Ingestion of sharp metallic objects may result in reticular perforation, localized or diffuse peritonitis, and in severe cases, extension to adjacent structures such as the diaphragm or pericardium.

Given the overlapping clinical presentation of TRP with other digestive disorders such as vagal indigestion, tympany, ruminal impaction, or intestinal obstruction, the diagnostic process remains complex. Traditional diagnostic approaches encompass clinical evaluation, imaging, hematology, and biochemical profiling. However, peritoneal fluid analysis has emerged as a rapid, minimally invasive, and highly informative diagnostic tool to evaluate peritoneal inflammation and infection.

Normal bovine peritoneal fluid was straw-colored, watery, and low in cellularity and protein (Radostits *et al.*, 2007) [8]. In contrast, inflammatory or septic peritonitis causes alterations such as increased turbidity, neutrophilic infiltration, elevated total protein, and the presence of fibrin, RBCs, and bacteria (Hussain & Uppal, 2014; Wittek *et al.*, 2010) [4, 11]. The diagnostic accuracy of cytological and biochemical analysis—especially D-dimer estimation—has been established in numerous studies, showing high sensitivity and specificity for septic peritonitis

(Wittek *et al.* 2010) ^[11]. Hussain *et al.* (2021) ^[6] further emphasized that cytology can detect degenerated neutrophils, engulfed bacteria, and fibrinous debris, which are essential for staging peritonitis.

In bovines, where systemic indicators may be less pronounced than in species like horses or dogs, peritoneal fluid analysis holds particular diagnostic value (Hussain *et al.*, 2021) ^[6]. Recent studies have expanded its application to diagnose complications such as perforated abomasal ulcers, intestinal obstruction, uterine torsion, and even reticular abscesses (Bhutia *et al.*, 2019; Brar *et al.*, 2006) ^[2, 3].

This study was designed to evaluate the cytological and d-dimer changes in peritoneal fluid in bovines affected with TRP and to establish a semi-quantitative scoring system for cytological evaluation to aid in diagnosis and clinical staging.

Materials and Methods

A total of six clinically diagnosed cases of traumatic reticuloperitonitis (TRP) in bovines were enrolled during the study period along with six healthy bovines during the period of March 2022 to November 2023 at Department of Veterinary Medicine, DGCN College of Veterinary and Animal Sciences, CSK Himachal Pradesh Agriculture University, Palampur, Himachal Pradesh, India.

Peritoneal Fluid Collection and Gross Evaluation

Peritoneal fluid was obtained via abdominocentesis from predetermined anatomical landmarks (Vermunt J, 2011) ^[10] or under ultrasonographic guidance in recumbent animals. Gross evaluation of the fluid included assessment of color, odor, turbidity, and volume.

Peritoneal Fluid Analysis

Peritoneal fluid samples collected in EDTA vials were analyzed using the Mindray BC-5000 Vet Hematology Analyzer for total leukocyte count (TLC), red blood cell (RBC) count, and neutrophil count (both absolute and differential). For D-dimer estimation, fluid was collected in 3.2% sodium citrate tubes and processed using the ICHROMA™ II analyzer. A 10 µl aliquot of the sample was mixed with the detection buffer, and 75 µl of the mixture was loaded into the cartridge for automated quantification. Results were generated within 12 minutes. Peritoneal fluid total protein concentration was estimated using a handheld refractometer.

Cytological Examination and Scoring

Cytological evaluation was performed on direct smears prepared from centrifuged peritoneal sediment. Smears were air-dried, methanol-fixed, and stained with Giemsa. Wet mount and stained preparations were examined under low (10×) and high-power (40× and 100× oil immersion) objectives. The following cytological parameters were evaluated: polymorphonuclear cells (PMNC), mononuclear cells (MNC), fibrin strands, bacteria, and RBCs.

Each parameter was semi-quantitatively scored based on visual estimation, averaging findings across 10 high-power (40×) microscopic fields. Scoring criteria were defined as follows:

PMNC and MNC (percentage of total nucleated cells/average of 10 high-power (40×) microscopic fields): 0 =

<10%, + = 10-30% (Mild), ++ = 31-60% (Moderate), +++ = >60% (Severe)

Bacteria and RBCs (relative abundance):

0 = Absent, + = Mild = ++, +++ = Marked

For statistical analysis, these categorical scores were converted to numerical values: 0 = 0, + = 1, ++ = 2, +++ = 3.

Tube Clot Test: Approximately 2-3 ml of peritoneal fluid was placed in a clean, dry glass test tube and left undisturbed at room temperature for 30 minutes. Clot formation indicated fibrin presence and was graded semi-quantitatively as + (thin film), ++ (moderate clot), or +++ (dense clot) (Radostits *et al.*, 2007 and Athar *et al.*, 2010) ^[1, 8].

Statistical Analysis

Quantitative data were expressed in Mean ± S.E. and analyzed using Welch's t-test. All statistical analyses were performed using GraphPad Prism version 9. Cytology scores and Tube clot scores from TRP and healthy control groups were analyzed using the Mann-Whitney U test due to the ordinal nature of the data and small sample size (n = 6 per group). Results were presented as median (range), and differences were considered statistically significant at $p < 0.05$.

Results

Gross appearance

Peritoneal fluid analysis in six bovines diagnosed with TRP (Table 1) revealed distinct deviations from normal findings. In terms of color, three animals (50%) exhibited yellow fluid, while the remaining three (50%) had brownish-red fluid (Fig. 1), indicating inflammatory and hemorrhagic reactions respectively. No cases showed straw-colored fluid, which was typical in healthy animals (Fig. 2).

Odour was absent in three animals (50%), while the other three showed a putrid smell, suggesting bacterial infection and tissue necrosis. The volume of peritoneal fluid was >50 ml in four animals (66.67%), consistent with inflammatory effusion, while two animals (33.33%) had fluid volumes <50 ml.

Turbidity was present in four animals (66.67%), further indicating active inflammation and exudate accumulation, whereas it was absent in the remaining two (33.33%).

Peritoneal Fluid Parameters

Various recorded peritoneal fluid parameters (Table 2) revealed significant alterations were observed in the peritoneal fluid profiles of TRP-affected bovines compared to healthy controls. Total red blood cell count was significantly higher in the diseased group ($p < 0.05$), indicating possible hemorrhagic effusion. Total leukocyte count and neutrophil count were markedly elevated in TRP cases ($p < 0.05$), with neutrophils comprising a significantly greater percentage of the leukocyte population ($p < 0.05$), consistent with a purulent inflammatory response. Total protein concentration was significantly elevated in TRP animals ($p < 0.01$), indicating an exudative nature of the effusion. D-dimer levels were also significantly higher in the diseased group compared to healthy controls ($p < 0.05$), reflecting ongoing fibrinolysis and inflammation.

Cytological Evaluation of Peritoneal Fluid

Cytological evaluation of bovines (Table 3) revealed normal peritoneal cytology characterized by mononuclear cell predominance (PMNC score: 0 to +) and absence of bacteria and red blood cells in healthy animals, establishing the baseline for comparison.

Among TRP cases, the mildest changes were observed in TRP 5, which showed mild neutrophilic infiltration without detectable bacteria or erythrocytes. This pattern was suggestive of early, or a sterile exudative response (Fig. 3).

Moderate cytological changes (Fig. 4) were noted in TRP 2 and TRP 4, marked by intracellular bacteria and mild to moderate hemorrhage, indicating mid-stage or localized peritonitis (Fig. 4).

Severe cytological alterations were present in TRP 1, TRP 3, and TRP 6, which showed marked (+++) polymorphonuclear cell infiltration, presence of intra- and extracellular bacteria, and a hemorrhagic background (Fig. 5 and Fig. 6). These findings were consistent with advanced septic TRP and exudative effusion (Fig. 5 and 6).

These stage-wise cytological patterns, when matched with reference Fig. images, enabled a clearer understanding of TRP progression from early sterile inflammation to advanced septic peritonitis.

Tube Clot Test Results

Peritoneal fluid samples subjected to the tube clot test (Table 3) demonstrated variable fibrin content. One sample showed a thin fibrin film (+), two samples exhibited moderate clot formation (++) (Fig. 7), and three samples formed dense gelatinous clots (+++), indicating substantial fibrin presence suggestive of ongoing or advanced inflammatory processes.

Cytological Score Comparison between Groups

Peritoneal cytology scores (Table 4) differed significantly between TRP-affected and healthy bovines. The PMNC score was significantly elevated in the TRP group compared to controls ($p = 0.0022$), indicating neutrophilic inflammation. Conversely, the MNC score was significantly reduced in affected animals ($p = 0.0022$), reflecting a shift from mononuclear to polymorphonuclear predominance. Bacterial presence, although absent in healthy bovines, was significantly increased in TRP animals ($p = 0.0152$), with both intracellular and extracellular organisms noted on smears. RBC scores were also significantly higher in the diseased group ($p = 0.0152$), suggesting hemorrhagic contamination or vascular leakage. These findings confirm the presence of septic, exudative peritonitis in TRP cases. Fibrin presence (Tube clot test) was also markedly higher in TRP cases ($p = 0.0022$), consistent with exudative inflammation.

A significant positive Spearman rank correlation was observed between tube clot scores and D-dimer concentrations in TRP-affected bovines ($\rho = 0.926$, $p = 0.033$), suggesting that greater fibrin deposition on cytology was associated with increased fibrinolytic activity in the peritoneal cavity.

Discussion

Peritoneal fluid analysis revealed significant alterations in bovines with TRP. Color changes from straw (healthy) to yellow and brownish-red in affected animals reflected the inflammatory and hemorrhagic nature of the effusion. The presence of turbidity and putrid odor in a majority of TRP cases suggests ongoing bacterial infection and necrosis, which aligns with the findings of Athar *et al.* (2010) [1] and Hussain *et al.* (2021) [6], who reported elevated neutrophils, degenerated PMNCs, and engulfed bacteria in peritonitis.

Significant increase in total protein ($p < 0.05$) confirmed the exudative character of peritoneal effusions in TRP, consistent with the classical differentiation of transudate and exudate described by Kopcha and Schultze (1991) [7] and Radostits *et al.* (2007) [8]. The markedly elevated leukocyte and neutrophil counts in diseased animals ($p < 0.05$) were suggestive of inflammation. These findings were further corroborated by elevated D-dimer levels, indicating active fibrinolysis, as previously validated by Wittek *et al.* (2010) [11].

Cytological scoring provided critical insights into disease severity. TRP animals showed significantly higher PMNC scores and reduced MNC scores ($p = 0.0022$), reflecting acute inflammatory progression (Fig. 3). Fibrin deposition, RBC infiltration, and bacterial presence were significantly higher in TRP animals ($p < 0.05$), supporting the findings of Hussain and Uppal (2014) [4] and Constable *et al.* (2017) [5], who observed degenerated neutrophils and engulfed bacteria in septic peritonitis.

These findings were also in agreement with Radostits *et al.* (2007) [8] and Constable *et al.* (2017) [5], who described severe peritoneal inflammation in TRP as being characterized by high protein, neutrophilic dominance, fibrin deposition, and bacterial presence. In the current study, gross fluid characteristics such as turbid appearance, putrid smell, and red-yellow discoloration corroborated the cytological and biochemical findings.

Peritoneal fluid analysis also enabled differential diagnosis from other causes of abdominal distress. While intestinal obstruction may present with high neutrophils and protein, bacterial presence and D-dimer elevations were more suggestive of septic TRP than ischemic or non-septic obstruction (Bhutia *et al.*, 2019) [2].

Table 1: Gross Characteristics of Peritoneal Fluid in Healthy Bovines and TRP Cases

Parameter	Observations	Healthy n=6	Traumatic reticulo-peritonitis (TRP) n=6
Colour	Yellow	-	3 (50%)
	Brownish Red	-	3 (50%)
	Straw	6 (100%)	-
Odour	Absent	6 (100%)	3 (50%)
	Putrid	-	3 (50%)
Volume	<50 ml	6 (100%)	2 (33.33%)
	>50 ml	-	4 (66.67%)
Turbidity	Present	-	4 (66.67%)
	Absent	6 (100%)	2 (33.33%)

Table 2: Comparative Analysis of Peritoneal Fluid Parameters in Healthy and TRP-Affected Bovines

Parameter	Healthy (n=6)	Diseased (n=6)
Total RBC ($\times 10^6/\mu\text{l}$)	0 $\pm$ 0	0.267 $\pm$ 0.067*
Total WBC ($\times 10^6/\mu\text{l}$)	0.95 $\pm$ 0.099	18.917 $\pm$ 4.183*
Neutrophils ($\times 10^6/\mu\text{l}$)	0.477 $\pm$ 0.052	15.167 $\pm$ 3.999*
Neutrophils (%)	50 $\pm$ 1.291	75 $\pm$ 5.323*
Total Protein (g/dl)	1.517 $\pm$ 0.06	4.55 $\pm$ 0.401**
D-dimer (mg/l)	0.40 $\pm$ 0.06	2.02 $\pm$ 0.6*

Values with * and ** in a row differs significantly ($P < 0.05$) and ($P < 0.01$) respectively from healthy animals

Table 3: Semi-quantitative Cytological Evaluation of Peritoneal Fluid in Healthy and TRP-Affected Bovines

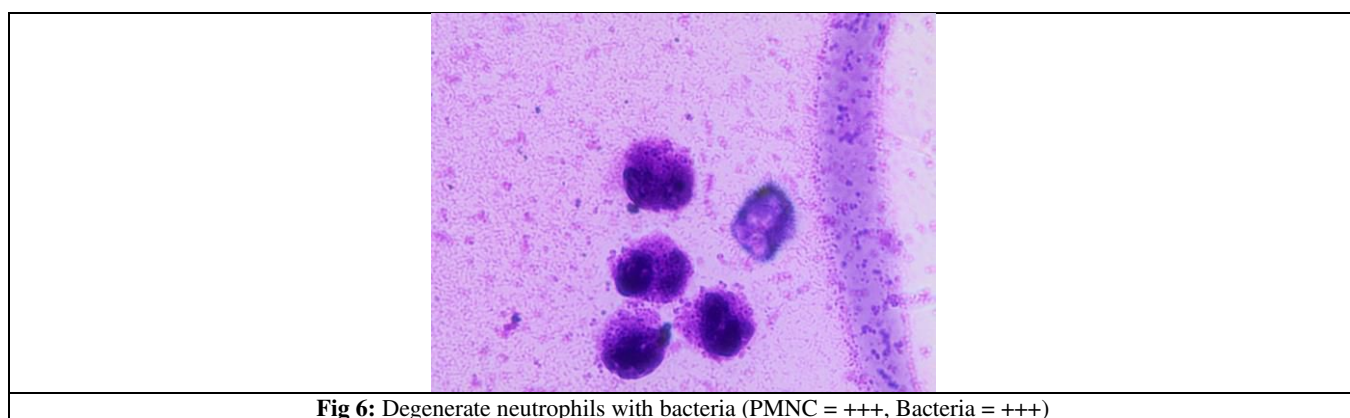
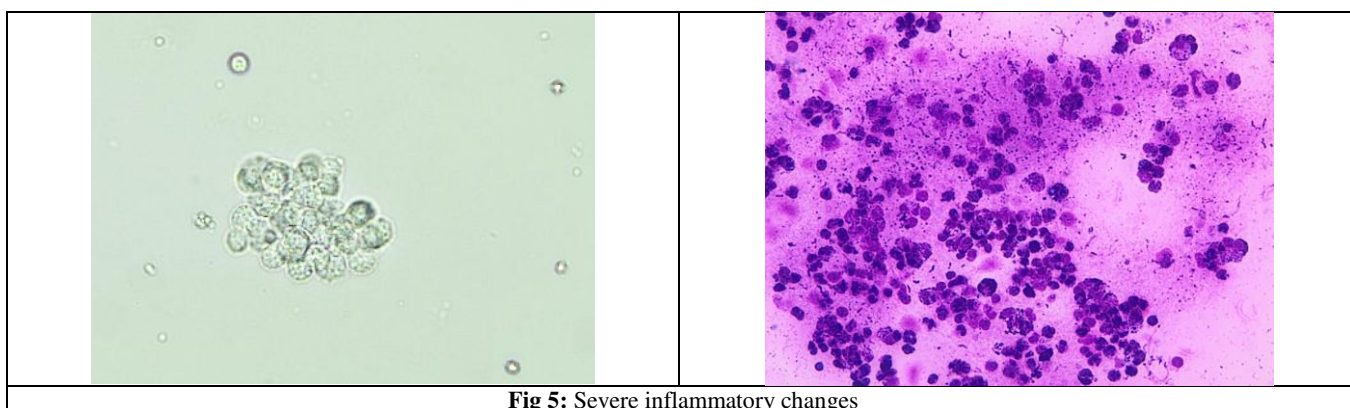
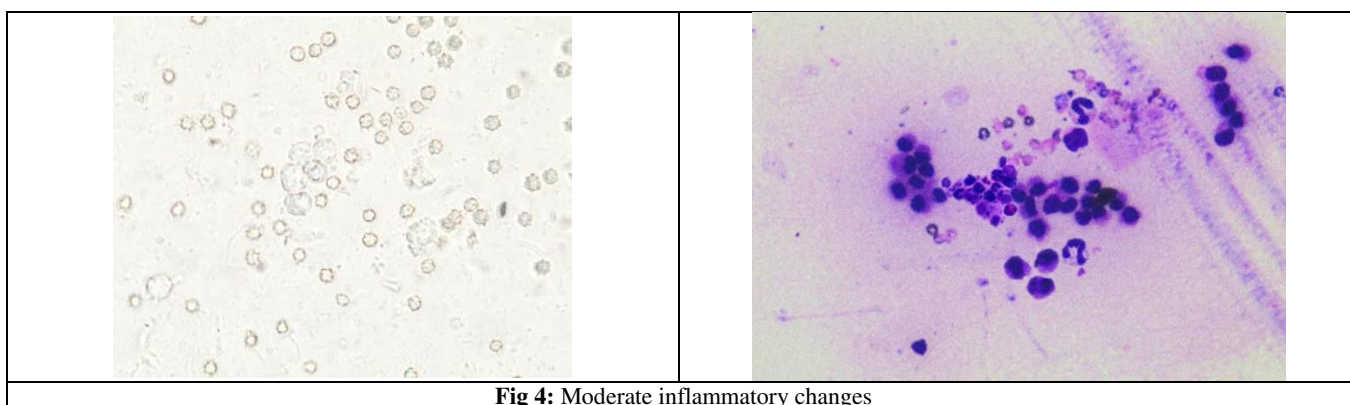
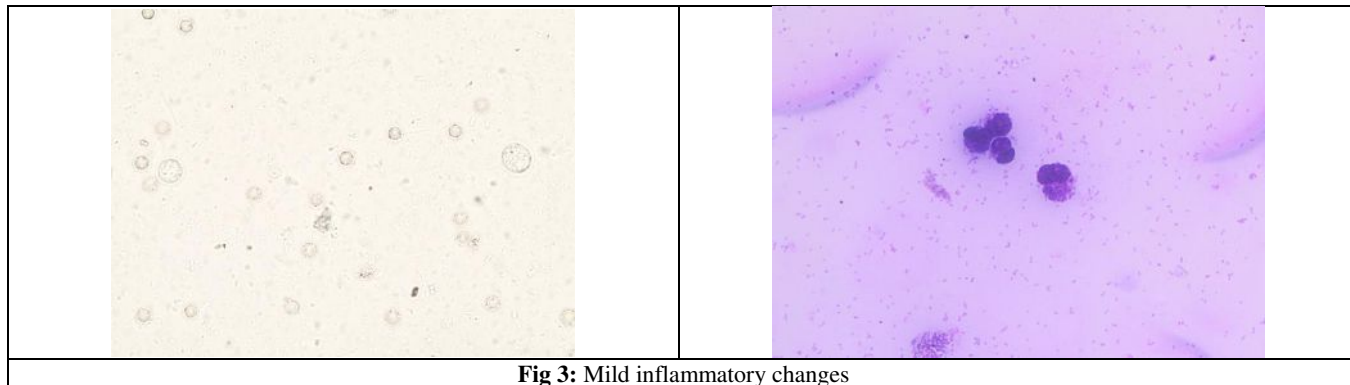
Animal	Cytological Scores	Severity	Tube Clot Test	Interpretation
Healthy 1-6	PMNC: 0-+, Bacteria: 0, RBC: 0	None (Normal)	0	Normal peritoneal cytology with mononuclear predominance, no inflammatory changes or contaminants. Serves as baseline for comparison.
TRP 5	PMNC: ++, Bacteria: 0, RBC: 0	Mild	+	Inflammation present without bacteria or RBCs; may represent sterile exudative response or early-stage inflammation.
TRP 2	PMNC: +++, Bacteria: + (intra), RBC: +	Moderate	++	Ongoing inflammation with intracellular bacteria and mild hemorrhage; suggestive of localized peritonitis.
TRP 4	PMNC: ++, Bacteria: +, RBC: +	Moderate	++	Moderate neutrophilic infiltration, presence of bacteria and mild RBCs, indicating mid-stage TRP.
TRP 1	PMNC: +++, Bacteria: ++, RBC: ++	Severe	+++	Marked neutrophilic infiltration, extracellular bacteria, and hemorrhagic background suggestive of septic peritonitis.
TRP 3	PMNC: +++, Bacteria: +++ (intra/extra), RBC: ++	Severe	+++	Classic signs of septic TRP with degenerate neutrophils and heavy bacterial load.
TRP 6	PMNC: +++, Bacteria: ++, RBC: ++	Severe	+++	High-grade inflammation with extracellular bacteria and hemorrhage confirms advanced septic exudative response.

Table 4: Comparison of Semi-Quantitative Cytological Scores & Tube clot scores Between Healthy and TRP-Affected Bovines (Mann-Whitney U-test)

Parameter	Median (Healthy)	Median (TRP)	p-value	Significance
PMNC	0.0	3.0	0.0022	**
MNC	3.0	1.0	0.0022	**
RBC	0.0	1.5	0.0152	*
Bacteria	0.0	1.5	0.0152	*
Tube clot score	0.0	2.5	0.0022	**

Values with * and ** in a row differs significantly ($P < 0.05$) and ($P < 0.01$) respectively from healthy animals

**Fig 1:** Brownish red coloured peritoneal fluid**Fig 2:** Normal peritoneal fluid



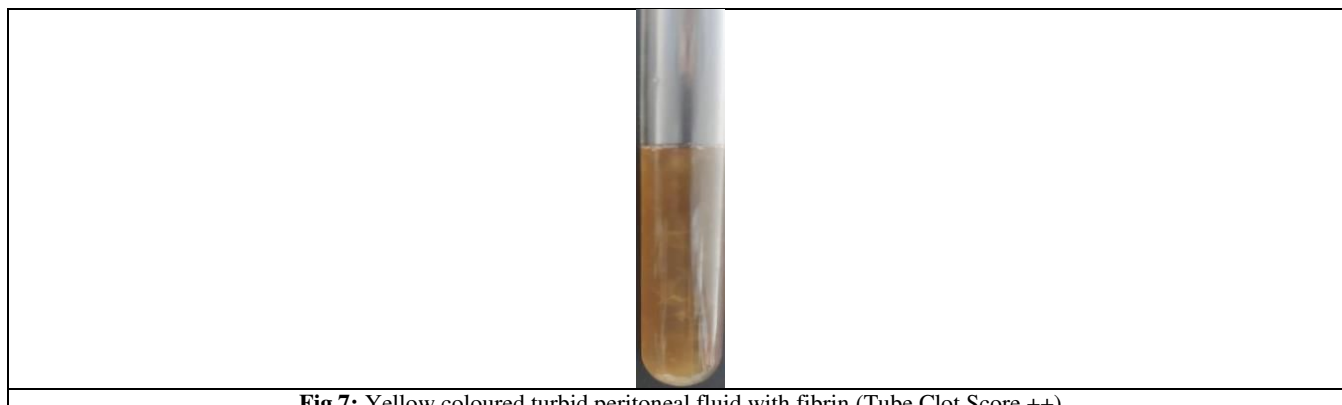


Fig 7: Yellow coloured turbid peritoneal fluid with fibrin (Tube Clot Score ++)

Conclusion

Integrated analysis of peritoneal fluid—through gross, biochemical, and cytological evaluation—provides a reliable diagnostic framework for traumatic reticuloperitonitis (TRP) in bovines. Gross examination indicated inflammatory or hemorrhagic effusion, biochemical tests quantified the exudative and fibrinolytic nature of the fluid, and cytology identified key inflammatory cells, their degeneration, and bacterial involvement.

Cytological evaluation proved especially valuable in field settings, using only basic tools like Giemsa stain and a microscope. The semi-quantitative scoring system developed enabled objective staging of TRP—from localized to septic peritonitis.

Combining modalities enhanced diagnostic sensitivity and specificity. Cytological findings correlated strongly with biochemical markers and tube clot scores, while the significant positive correlation between tube clot scores and D-dimer levels confirmed their complementary diagnostic role. Overall, peritoneal fluid cytology emerged as a rapid, minimally invasive, and practical tool for early TRP diagnosis, particularly in resource-limited field conditions.

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