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Diagnostic relevance of rumen chloride and protozoal activity in gastrointestinal disorders in cattle

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Abstract
The presented study was aimed at comparing the clinical signs, hemato-biochemical changes and rumen fluid analysis, with an emphasis on ruminal chloride concentration, in major gastrointestinal (GIT) disorders of cattle. Forty-eight cattle were categorized into different groups like enteritis, ruminal alkalosis, bloat, intestinal obstruction, and ruminal impaction, along with six healthy cattle that were taken as control. Anorexia, rumen stasis, reduced fecal output and dehydration were the most common clinical signs presented in majority disorders. Rumen pH was significantly elevated in ruminal alkalosis (8.28 ± 0.15) and ruminal impaction (8.17 ± 0.18) ($p<0.01$), while rumen protozoa motility was significantly reduced in all groups except for bloat and enteritis. Mann-Whitney U-test revealed significantly low rumen protozoa scores in ruminal alkalosis, intestinal obstruction, and ruminal impaction ($p<0.01$). Rumen chloride concentration was significantly reduced in ruminal alkalosis (15.2 ± 1.13 mEq/L) but significantly elevated in intestinal obstruction (42.53 ± 6.39 mEq/L; $p<0.05$). These findings highlighted the diagnostic value of rumen fluid analysis, especially rumen chloride concentration and ruminal protozoa motility, in differentiating among various GIT disorders in cattle.

Keywords: Gastrointestinal disorders, ruminal chloride, hemato-biochemical profile, cattle

Introduction

Gastrointestinal (GIT) disorders are major cause of economic losses in cattle globally (Enemark, 2008; Plaizier *et al.*, 2009; Steele *et al.*, 2009) [12, 27, 29]. Disorders such as enteritis, ruminal alkalosis, bloat, intestinal obstruction, and ruminal impaction often presented with nonspecific signs like anorexia, reduced milk yield, abdominal distension, altered rumen motility, and dehydration (Radostits *et al.*, 2007) [28]. These overlapping clinical signs of GIT disorders make diagnosis challenging (Desiye and Mersha, 2012; Kahn, 2011) [10, 20].

Ruminants are exclusively capable of digesting plant cell wall polysaccharides i.e. cellulose, hemicellulose, and pectin; converting them into meat and milk (Bradford *et al.*, 2003) [4]. However, this fermentation process depends on the functional and microbial integrity of the rumen. Disruption in rumen health leads to indigestion, metabolic imbalances, reduced feed conversion efficiency, and ultimately, financial loss (Kirbas *et al.*, 2014) [22].

Many GIT disorders arise from poor-quality feed or abrupt dietary transitions, triggering syndromes like bloat, ruminal alkalosis, or ruminal impaction (Radostits *et al.*, 2007) [28]. Field diagnosis is often based on clinical signs, which may be insufficient due to their overlapping nature across various conditions. Therefore, rumen fluid analysis has gained prominence in the diagnostic algorithms.

Among rumen fluid parameters, chloride concentration is a sensitive biomarker. Elevated ruminal chloride concentration suggests abomasal or duodenal reflux due to obstructions (Braun *et al.*, 2024) [5], while lower levels may indicate excessive salivation or absorption disturbance, as observed in ruminal alkalosis. Dirksen *et al.* (2006) [11] similarly associated high ruminal Cl^- with abomasal reflux secondary to intestinal blockage.

Additionally, rumen protozoal activity offers insight into the fermentative status of the rumen. A decline in count and motility of protozoa may reflect microbial depression resulting from pH imbalance, starvation, or abomasal reflux.

This study aims to study, evaluate and compare the clinical, hematobiochemical, and ruminal fluid profiles-particularly chloride concentration and protozoal count and motilitycin cattle affected with major GIT disorders.

Materials and Methods

A total of forty-eight cattle diagnosed with various gastro-intestinal disorder were enrolled during the study period along with six healthy cattle during the period of June 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.

The affected cattle were categorized into five groups based on clinical signs and diagnostic findings: enteritis (n = 16), ruminal alkalosis (n = 6), bloat (n = 14), intestinal obstruction (n = 6), and ruminal impaction (n = 6). All cattle underwent thorough clinical examination including rectal temperature, heart and respiratory rates, rumen motility, and fecal consistency. Blood samples were collected aseptically for hematological and serum biochemical analysis. Serum electrolyte concentrations of sodium (Na^+), potassium (K^+), and chloride (Cl^-) were measured using an automated electrolyte analyzer (CB Lyte, Chariot's Biotechnology, Mumbai, India).

Rumen fluid (5-10 mL) was collected aseptically from the left paralumbar fossa using a 16G, 6-inch hypodermic needle (Kiro, 2017). Physical characteristics (color, odor, consistency) were assessed, and pH was measured using pH paper and a digital meter. Microscopic examination for protozoal motility was performed and scored semi-quantitatively (0 to 3 scale) as per Chakrabarti (2006). Rumen fluid was centrifuged at 3000 rpm for 5 minutes, and the supernatant was analyzed for electrolyte concentrations. Rumen chloride estimation was done using the CB Lyte analyzer, with levels >30 mmol/L interpreted as indicative of abomasal reflux or ileus. Statistical analysis was performed using ANOVA and Mann-Whitney U-test. Results were presented as Mean ± Standard Error, with significance set at $p< 0.05$ and $p< 0.01$.

Results and Discussion

The present study evaluated clinical, hemato-biochemical, electrolyte, and rumen fluid changes in various gastrointestinal (GIT) disorders in cattle i.e. enteritis, ruminal alkalosis, bloat, intestinal obstruction, and ruminal impaction.

Clinical signs recorded were anorexia and absence of water intake, majorly in ruminal alkalosis (66.67%), bloat (71.43%), and intestinal obstruction (83.33%). Rumination was absent in all cattle affected with ruminal alkalosis and reduced in bloat (66.67%) and obstruction (83.33%). Reduced fecal output was noted in bloat (78.57%) and intestinal obstruction (83.33%), while increased defecation was observed in all cattle affected with enteritis. Weak body condition and dullness were observed in enteritis and intestinal obstruction (66.67%). Abdominal signs like looking at flank (78.57%) and severe left flank distension (64.29%) were typical of bloat. Fecal character like scanty, mucus-laden feces were observed in intestinal obstruction, while ruminal impaction cases had hard and mucoid feces.

An increased heart and respiratory rates was observed in cattle affected with bloat and obstruction, which could be due to diaphragmatic compression and systemic hypoxia. These findings were in alignment with the findings of Constable *et al.* (2017a) [7]. Heart rate was within normal limits in cattle with ruminal alkalosis and ruminal impaction (Constable *et al.*, 2017b) [8]. Rumen motility was reduced in bloat and ruminal impaction. These findings were consistent with the findings of Braun *et al.* (2024) [5] and Athar *et al.* (2010a) [2]. Rumen contractions were moderately reduced by enteritis and ruminal alkalosis.

The haematological, biochemical and electrolyte changes of cattle affected with various gastrointestinal disorder are presented in Table 1. Haematological findings revealed leukocytosis and neutrophilia in enteritis, bloat, and intestinal obstruction, suggestive of inflammation, while lymphopenia in intestinal obstruction and ruminal impaction indicated stress. These findings were similar to the findings of Hassan *et al.* (2022) [17], Hailat *et al.* (1996) [16], and Feldman *et al.* (2000) [13].

Biochemical alterations included significantly elevated AST in ruminal alkalosis, intestinal obstruction, and ruminal impaction; indicating hepatic stress or muscle catabolism (Garry, 2002; Kaneko *et al.*, 2008) [14, 21]. Total protein concentration was decreased in enteritis and ruminal impaction, likely due to anorexia, protein-losing enteropathy, and reduced protein absorption, whereas it was elevated in intestinal obstruction due to dehydration leading to haemoconcentration and inflammatory protein response. Elevated BUN and blood glucose were seen in most disorders, suggesting prerenal azotemia and stress-induced hyperglycemia and were in alignment with the findings of Avery *et al.*, 1986; Braun *et al.*, 2023; Mudroň *et al.*, 2005 [3, 6, 26]. Electrolyte imbalances included hyponatremia in enteritis and hypernatremia in ruminal alkalosis. Hypokalemia was seen in intestinal obstruction and ruminal impaction. Chloride was significantly reduced in enteritis, ruminal alkalosis, and intestinal obstruction. These findings were similar to that of Aref *et al.* (2017) [1] and Kaneko *et al.* (2008) [21].

Table 1: Haemato-biochemical & Electrolyte changes in cattle with Gastrointestinal Disorder

Parameter	Healthy	Enteritis	Alkaline indigestion	Bloat	Intestinal obstruction	Ruminal impaction
	n=6	n=26	n=6	n=14	n=6	n=6
TLC ($X10^3/\mu L$)	7.84±0.24	10.09±0.56**	8.85±0.48	11.41±1.39*	12.02±1.37*	9.1±0.55
Neutrophils (%)	28.33±0.73	34.87±2.13**	27.81±1.94	47.91±6.64*	52.93±7.84*	41.33±4.77*
Lymphocytes (%)	64.55±0.78	59.54±2.16**	63.85±2.16	46.78±8.01*	40.72±9.09*	54.45±4.35
Hb (g/dL)	10.9±0.48	9.54±0.30	10.59±0.54	12.76±0.91	11.90±0.30	12.5±0.98
HCT (%)	33.92±1.55	38.78±2.08	35.46±2.64	41.13±3.31	42.34±1.52**	40.22±1.46*
MCV (fL)	47.49±1.66	49.67±0.74	49.51±1.55	47.73±2.64	48.72±4.87	46.38±2.48
MCH (pg)	16.02±0.85	15.78±0.38	16.78±0.35	17.91±0.63	17.16±0.71	16.02±0.49
AST/SGOT (U/L)	80.23±3.31	84.22±6.80	128.49±11.24**	83.01±6.07	167.63±20.77**	170.19±32.13*
GGT (U/L)	13.98±0.81	15.73±1.15	18.7±1.46**	16.04±1.13	22.51±3.84	35.71±19.58
BIL (mg/dL)	0.49±0.05	0.54±0.05	0.54±0.06*	0.45±0.06	1.08±0.15*	0.94±0.20
TP (mg/dL)	7.53±0.15	6.54±0.10**	7.23±0.23	7.15±0.26	8.62±0.37*	6.46±0.29*
BUN (mg/dL)	18.92±0.81	19.81±0.76	23.91±1.29**	23.7±2.56	47.34±9.55*	51.59±3.73**
GLU (mg/dL)	53.73±1.36	60.87±2.54*	61.43±2.65*	64.00±3.69*	98.89±12.78*	89.70±1.89**
Na^+ (mEq/L)	144.82±3.11	133.31±2.06*	154.54±2.89*	144.11±1.13	140.44±2.94	138.23±2.18
K^+ (mEq/L)	4.52±0.1	4.08±0.12*	3.98±0.26	4.33±0.18	3.45±0.17**	3.76±0.22*
Cl^- (mEq/L)	102.53±0.95	97.5±0.54**	97.49±1.71*	100.47±0.76	85.94±2.22**	98.7±1.58

Rumen fluid analysis of cattle affected with various gastro-intestinal disorder are presented in Table 2. Rumen fluid analysis revealed that rumen pH was significantly elevated in ruminal alkalosis and ruminal impaction, while color changes (yellow-brown) and watery consistency were most pronounced in ruminal alkalosis, intestinal obstruction, and ruminal impaction. Enteritis and bloat maintained relatively normal rumen fluid characteristics. Protozoal motility was markedly reduced in ruminal alkalosis, intestinal obstruction, and ruminal impaction, consistent with microbial depression (Aref *et al*., 2017) [1].

Table 2: Rumen fluid examination in cattle with Gastro-intestinal disorder

Parameter Observations	Healthy	Enteritis	Alkaline indigestion	Bloat	Intestinal obstruction	Ruminal impaction
	n=6 (Mean ± S.E)	n=16 (Mean ± S.E)	n=6 (Mean ± S.E)	n=14 (Mean ± S.E)	n=6 (Mean ± S.E)	n=6 (Mean ± S.E)
pH	6.67±0.13	7.10±0.19	8.28±0.15**	6.46±0.15	6.67±0.42	8.17±0.18**
Colour						
Green	6(100%)	11(68.75%)	-	14 (100%)	2(33.33%)	1(16.67%)
Gray	-	-	-	-	-	-
Yellowish brown	-	5(31.25%)	6 (100%)	-	4(66.67%)	5(83.33%)
Consistency						
Normal/Slightly Viscous	6(100%)	16(100%)	-	14(100%)	2(33.33%)	2(33.33%)
Watery	-	-	6 (100%)	-	4(66.67%)	4(66.67%)
Protozoa motility						
±	-	-	-	-	-	-
+	-	-	6 (100%)	-	5 (83.33%)	3(50%)
++	1 (16.67%)	5(31.25%)	-	2(14.29%)	1 (16.67%)	3(50%)
+++	5 (83.33%)	11(68.75%)	-	12(85.71%)	-	-

Protozoal motility was significantly reduced in cases of ruminal ruminal alkalosis, intestinal obstruction, and ruminal impaction, as confirmed by the Mann-Whitney U-test ($p < 0.01$; Table 3). The uniformly minimal motility observed in ruminal alkalosis cases likely reflects a hostile rumen environment due to elevated pH, which impairs protozoal viability. Reduced protozoal activity in cases of intestinal obstruction and ruminal impaction could be due to prolonged stasis, nutrient deprivation, and altered rumen fermentation. Conversely, enteritis and bloat did not significantly affect protozoal motility, suggesting these conditions had less severe or transient impacts on protozoal function.

Table 3: Mann-Whitney U-test Comparison of Protozoal Motility

Comparison Group vs. Healthy	Median (Group)	Median (Healthy)	U-value	p-value	Significance
Enteritis	3.00	3.00	41.00	0.6341	ns
Ruminal alkalosis	1.00	3.00	0.00	0.0022	** ($p < 0.01$)
Bloat	3.00	3.00	41.00	>0.9999	ns
Intestinal Obstruction	1.00	3.00	0.50	0.0043	** ($p < 0.01$)
Ruminal Impaction	0.50	3.00	0.00	0.0022	** ($p < 0.01$)

Rumen fluid electrolyte analysis (Table 4) showed a significant decrease in sodium levels in ruminal impaction cases ($p<0.01$), which could be due to reduced intake, prolonged stasis, and intracellular electrolyte shifts, corresponding to the findings of Aref *et al*. (2017) [1]. Potassium concentration was also decreased in both ruminal alkalosis ($p<0.01$) and ruminal impaction ($p<0.05$), indicating depletion caused due to anorexia, decreased rumen turnover, and intracellular redistribution, similar to observations noted by Kaneko *et al*. (2008) [21]. A marked elevation in ruminal chloride was observed in intestinal obstruction, indicating abomasal reflux (Braun *et al*., 2024) [5], whereas chloride levels were significantly reduced in ruminal alkalosis ($p<0.05$), that could be due to excessive salivary dilution and impaired mucosal absorption (Dirksen *et al*., 2006) [11].

Table 4: Rumen Fluid Electrolyte Profile

Parameter	Healthy (n=6)	Enteritis (n=16)	Alkaline indigestion (n=6)	Bloat (n=14)	Intestinal Obstruction (n=6)	Ruminal Impaction (n=6)
Na^+ (mEq/L)	141.37±3.5	139.8±5.47	155.9±3.41*	138.87±2.33	148.48±4.36	124.64±3.5**
K^+ (mEq/L)	36.48±2.32	34.53±1.36	27.05±0.29**	31.58±2.12	30.38±2.24	29.14±1.57*
Cl^- (mEq/L)	22.27±2.13	21.74±1.45	15.2±1.13*	20.25±1.2	42.53±6.39*	18.75±2.76

These findings highlight the diagnostic importance of combining clinical assessment with hematobiochemical changes and rumen fluid evaluation-particularly pH, protozoal activity, and chloride concentration-for differentiating GIT disorders in cattle under field conditions. The varying degrees of protozoal motility observed (+ and +++) are represented in Fig. 1 and Fig. 2, respectively.

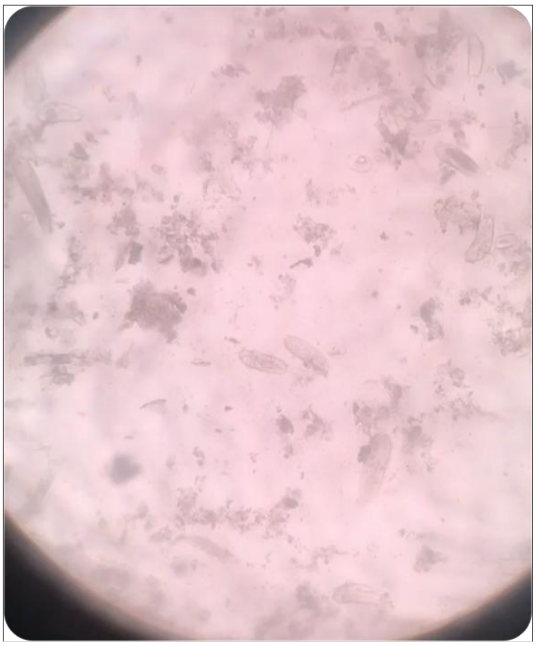

Fig 1: Microscopy of Ruminal fluid in Ruminal Impaction (Protozoa motility score: +)

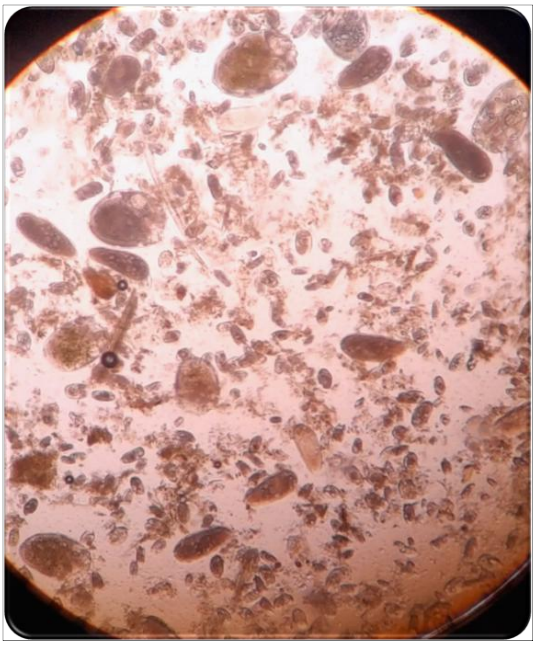

Fig 2: Microscopy of Healthy Rumen Fluid (Protozoa motility: +++)

Conclusion

This study presents a comprehensive clinical, hematobiochemical, and rumen fluid assessment of major gastrointestinal disorders in cattle-enteritis, ruminal alkalosis, bloat, intestinal obstruction, and ruminal impaction. Although each condition has distinct features, considerable overlap in clinical signs made differentiation difficult using physical examination alone. Hematological alterations, particularly leukocytosis with neutrophilia and lymphopenia, were consistent indicators of systemic inflammation and stress, while serum biochemical changes (elevated AST, ALP, bilirubin, BUN, and glucose) supported diagnoses related to dehydration, hepatic involvement, and metabolic stress.

Electrolyte alterations displayed condition-specific patterns, mirroring the underlying fluid redistribution and metabolic responses associated with each disorder. Amongst all diagnostic approaches, rumen fluid analysis proved to be the most sensitive tool, with variations in pH, protozoal motility, and particularly ruminal chloride concentration offering clear distinction between different disorders. Increased rumen chloride levels were strongly linked to abomasal reflux in cases of intestinal obstruction, while reduced chloride concentrations were characteristic of ruminal alkalosis. Statistical evaluation using the Mann-Whitney U-test reinforced the diagnostic significance of both protozoal counts and chloride levels across the disorders examined.

Furthermore, incorporating rumen microbiome profiling and advanced metabolic biomarkers could further strengthen diagnostic accuracy and enable earlier, more targeted therapeutic interventions for cattle affected with gastrointestinal diseases.

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