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Original Article

Primary type 3 abomasal ulceration in cattle and buffalo: clinico-biochemical parameters, treatment, and prognostic indicators

Hussain, S. A.[1, 2*]; Uppal, S. K.[2]; Sood, N. K.[3] and Mohindroo, J.[4]

[1]Division of Clinical Veterinary Medicine, Ethics and Jurisprudence, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, Jammu and Kashmir-190006, India; [2]Department of Veterinary Medicine, College of Veterinary Sciences, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, Punjab 141004, India; [3]Department of Teaching Veterinary Clinical Complex, College of Veterinary Sciences, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, Punjab 141004, India; [4]Department of Veterinary Surgery and Radiology, College of Veterinary Sciences, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, Punjab 141004, India

[*]***Correspondence:*** *S. A. Hussain, Division of Clinical Veterinary Medicine, Ethics and Jurisprudence, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, Jammu and Kashmir-190006, India. E-mail: draashiqhussain@gmail.com*

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Abstract

Background: The clinical findings, laboratory alterations, and prognosis of primary type 3 abomasal ulcer (AU3) are poorly reported in the literature. **Aims:** To describe clinical findings, hemato-biochemical changes, and peritoneal fluid changes in bovines suffering from primary AU3, and to monitor responses to medical treatment and outcomes. **Methods:** The study included 32 bovines (20 cattle and 12 buffaloes) diagnosed with primary AU3 along with a control group. **Results:** Common clinical findings were depressed demeanor, anorexia, dehydration, scanty feces, melena, mushy atonic rumen, tachycardia, and tachypnea. Colic was observed in 56.3% of animals. The mean hemoglobin, hematocrit, platelet count, and lymphocyte count were lower ($P \leq 0.05$), while WBC and neutrophil count were higher than the values of the control group ($P \leq 0.05$). The levels of BHBA, NEFA, glucose, total bilirubin, AST, CK, LDH, BUN, creatinine, and lactate were higher ($P \leq 0.05$), while cholesterol, total protein, albumin, sodium, potassium, chloride, and calcium were lower than the values of the control group ($P \leq 0.05$). The rumen chloride concentration was increased. The left shift was observed in a higher percentage of nonsurvivors than survivors ($P \leq 0.05$). The nonsurvivors had higher levels of bilirubin, CK, LDH, BUN, creatinine, and rumen chloride ($P \leq 0.05$), and lower levels of total protein, albumin, and globulin ($P \leq 0.05$). **Conclusion:** Type 3 abomasal ulcers occurred during the various stages of lactation as well as in pregnant animals. The response to medical treatment was fair, long time survival rate was good, and there was no recurrence. There was no effect on fetal survival or milk yield in the subsequent lactation.

Key words: Biochemistry, Peritoneal fluid, Survival, Therapy, Type 3 abomasal ulcer

Introduction

Abomasal ulcers, an important cause of indigestion in bovines, can be primary or secondary to other diseases (Constable *et al*., 2017). Abomasal ulcers have been categorized into four (Whitlock, 1980; Smith *et al*., 1983) or five (Constable, 2010) types, each with distinct pathomorphological features. Type 3 abomasal ulcer (AU3) is a perforated abomasal ulcer with localized peritonitis (Braun *et al*., 2019). The clinical signs of AU3 have been reported to resemble to that of traumatic reticuloperitonitis (TRP) and TRP is the main disease for differential diagnosis of AU3 (Francoz and Guard, 2015; Constable *et al*., 2017; Braun *et al*., 2019). As per Whitlock (1980), melena is not a typical feature of AU3 as abomasal hemorrhage is minimal but Smith *et al*. (1983) reported melena in 80% of cows with AU3. The diagnosis of AU3 is usually based on clinical evaluation after ruling out TRP (Constable, 2010). On ultrasonography, the peritoneal changes in the anterior abdomen are considered to be suggestive of AU3 provided that TRP is ruled out (Braun, 2009). The treatment of AU3 per se should be aimed at increasing the abomasal pH and decreasing the secretion of hydrochloric acid and pepsin. As hypocalcemia is not uncommon in bovines with gastrointestinal disorders (Fecteau *et al*., 2018; Hussain *et al*., 2021a, 2022), treatment of AU3 with calcium may be intuitive.

The primary AU3 has not been reported in buffaloes. The available clinical reports on AU3 in cattle involve

either small number of cases (Smith *et al*., 1983; Palmer and Whitlock, 1984; Cable *et al*., 1998) or few laboratory parameters (Braun *et al*., 2019). The reported hemato-biochemical parameters of AU3 involve only a few parameters i.e. hematocrit, white blood cell count, total protein, fibrinogen, urea, potassium, and chloride (Palmer and Whitlock, 1984; Braun *et al*., 2019). Peritoneal fluid changes are reported in 4-5 cows in different studies (Cable *et al*., 1998; Braun *et al*., 2019). Further, the available literature is mainly about secondary AU3 and the data about primary AU3 is lacking. The information about biochemical alterations in primary and secondary AU3 is scarce. Data about energy and lipid metabolism parameters are lacking, and the data about peritoneal fluid alterations and treatment of AU3 are also limited (Braun *et al*., 2019). The effect of AU3 on milk production has not been evaluated previously, and data about prognostic indicators are lacking. Therefore, the present study was undertaken to describe clinical findings, hemato-biochemical changes, peritoneal fluid changes, response to medical treatment, and outcome for bovines suffering from primary AU3. Another objective was to determine prognostic factors associated with the outcome of the disease, milk production in the current and subsequent lactation, and long-term outcome.

Materials and Methods

Animals and diagnosis of AU3

The study was performed in compliance with the institutional ethical guidelines. The study included 32 bovines (20 cattle and 12 buffaloes) suffering from AU3. The diagnosis of AU3 was on the basis of clinical examination, abnormal abdominocentesis, and ultra-sonography (Belknap and Navarre, 2000; Constable, 2010; Tharwat and Ahmed, 2012). The main diagnostic criterion was localized peritonitis with evidence of gut content leakage in the peritoneal fluid examination. Before predicting the diagnosis, other important causes of localized peritonitis i.e. TRP was ruled out by physical examination and other ancillary tests including ferroscopy, radiography and ultrasonography. None of these animals had evidence of any other diseases. In eight animals, the diagnosis was further confirmed on necropsy examination.

A detailed history was recorded by an interview with the owner. Each animal was subjected to a general physical examination and a special examination of the gastrointestinal system (Rosenberger, 1990; Hussain *et al*., 2013).

Laboratory analyses

The blood samples, collected in EDTA coated vials, were used for the determination of hematological parameters. Hematological parameters, except differential cell count, were estimated by an automatic hematology analyzer (ADVIA® 2120 Hematology system, Siemens Healthcare Diagnostics Inc., USA). Differential leukocyte count was performed manually in the blood smear stained by the Leishman stain. Serum was used for the measurement of biochemical parameters except glucose, and fibrinogen. For glucose and fibrinogen estimation, blood samples were collected in sodium fluoride and sodium citrate coated vials, respectively. β-hydroxybutyrate (BHBA) and non-esterified fatty acids (NEFA) were determined by a kits manufactured by DiaSys Diagnostic systems (India). Fibrinogen was measured using commercially available reagents (Tulip Diagnostics (P) Ltd., Goa, India) as per the manufacturer's guidelines by the Sysmex CA/50 blood coagulation analyzer (Sysmex Corporation, Kobe, Japan). The VITROS 350/250/250AT Chemistry system (Ortho-Clinical Diagnostics, Johnson and Johnson Co.) was used for the measurement of other biochemical parameters.

Rumen liquor samples were collected, then pH and chloride concentrations of them were evaluated, as described by Hussain *et al*. (2013). Abdominocentesis was done at the post-xiphoid site in all the animals (Hussain *et al*., 2021b). The samples of peritoneal fluid were collected in the EDTA coated vials. The volume, color, consistency/turbidity, and specific gravity of peritoneal fluid were recorded for each case. Specific gravity was measured using a handheld refractometer, and total leukocyte and neutrophil counts were determined as described for blood samples. Total protein was estimated by the same method used for blood. Paracentesis of the abomasum was carried out using a 16-gauge needle to determine the pH of the aspirated fluid (Braun *et al*., 1997; Constable *et al*., 2017).

Fecal samples were tested for occult blood by HEMOSPOT test cards (Crest Biosystems, A Division of Coral Clinical Systems, Goa, India) based on the standard Guaiac method (Hussain *et al*., 2015a).

Radiography, ferroscopy, and ultrasonography

Reticular radiographs were taken in 29 animals as described by Hussain *et al*. (2021a). All animals were subjected to ferroscopic examination (Hauptner Ferrsocope, Art-Nr 39500; H. Hauptner & Richard Herberholzgmb H & Co., KG, Solingen, Germany). The ultrasonography of abdomen was performed with Wipro GE Logiq III Expert/Aloka 500V in real time B mode and B+M mode by a standard procedure (Braun, 2009).

Treatment and follow up

Three animals died on the day of presentation, and the 29 animals were treated with antibiotics, antacids, and other supportive treatments. Depending upon the severity of the hemato-biochemical alterations, the antibiotics were administered. Treatment included intravenous administration of 5-10 L of normal saline and 5 L of dextrose normal saline for 3-5 days, 1 dose of intravenous calcium therapy (450 ml of mifex, Novartis India Limited, India), 200 ml of liquid potklor (containing 20 g of potassium chloride) orally for 3 days, 10 ml intramuscular injection of rumeric (B complex and amino acids) for 3-8 days, 10 ml subcutaneous injection

of repronol (each ml containing 50 mg vitamin E and 1.5 mg of sodium selenite) for 3 days, and 100 g charcoal (as anti-bloat agent) once daily till resolution of tympany. Oral antacid (magnesium oxide, 200-400 g per animal per day) or parenteral antacid (ranitidine, 3 mg/kg bid) was administered till resolution of melena. The animals were discharged on the third day of treatment. Follow-up information was obtained from the owners by telephonic conversation. The owners were contacted at 3 day intervals for 15 days, weekly for 2 months, then at a regular interval of three months for a period of 24 months. The owners were questioned about the general health status of the animals, and the born calves (for pregnant animals), any effect on milk yield in the current and subsequent lactation, and any recurrence of disease in the next pregnancy.

Control group

Ten cows and ten buffaloes from the university dairy farm served as the control group. All these animals were healthy at the time of sampling, between the first to third lactation, and had a negative fecal occult blood test.

Statistical analysis

Student's t-test was used to indicate the significant differences between AU3 and control groups, and between survivors and non survivors. Chi-square test was used for the comparison of binary variables between survivors and non survivors. The data were analysed by SPSS, ver. 20 and significance level was $P \leq 0.05$ for all statistical procedures.

Results

Historical and clinical findings

The animals suffering from AU3 were 2-10 years old females (mean=5.23±0.35, median=5 years). Seventy two percent of animals were in their 1st-2nd lactation (n=18) or heifers (n=5), six were in third lactation, two in 5th and one was in 6th lactation. The majority of the animals (n=19/32) were non-pregnant and 13 were in different stages of pregnancy (Fig. 1).

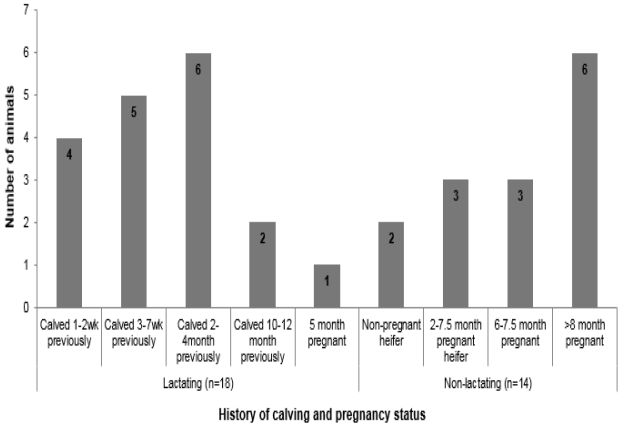

Fig. 1: Frequency of type 3 abomasal ulcers with respect to calving and pregnancy status

The duration of the illness (mean 10.3±1.22, median=8 days) was less than a week in ten, 1-2 weeks in fourteen, 2-3 weeks in six, and 30 days in two animals. The historical and clinical examination findings are presented in Tables 1 and 2. The majority of animals had a history of sudden anorexia or fever followed by scanty defecation. There was a history of melena in all, except two animals. Milk yield was suddenly fallen in 78% of the lactating animals. There was a history of colic in 16 animals that was persistent in seven, intermittent in four, and only observed for initial 3-4 days in five animals. Additionally, the signs of colic were observed on clinical examination of two animals with no previous history of colic. Before referral to our clinic, all except two animals had been treated symptomatically.

Table 1: Historical findings in 32 bovines with primary type 3 abomasal ulcer

Characteristic	Finding	Number of animals
Feed intake	Reduced	03
	Absent	29
Water intake	Normal	11
	Reduced	17
	Absent	04
History of fever	Present	20
	Absent	12
History of abdominal pain	Present	16
	Absent	16
History of tympany	Persistent	06
	Absent	23
	Once	03
Reduction in milk yield*	Sudden	14
	Gradual	04
Defecation	Loose/diarrhea**	05
	Scanty	20
	Absent	05
	Normal	02

* Only 18 animals were lactating, and ** Diarrhea followed by the loss of defecation in one and diarrhea followed by scanty feces in one

Per rectal examination revealed black color scanty quantity of pasty consistency feces in the majority of the animals (Table 3) and mild caecal dilatation in four animals. Pain on post xiphoid palpation (mainly right side) was noted in 15 animals, pain on right post xiphoid palpation along with a positive wither pinch test in five animals, and a positive wither pinch test in three other animals. Physical examination revealed tympany in two animals with no previous history of tympany. Fecal occult blood test was positive in 29 out of 30 tested animals. Physical examination revealed pneumo-peritonium in six animals, ruminal ping in three, right side ping in one animal, and fluid splash on swinging palpation of the abdomen in four animals. Two animals had left-side abdominal ping similar to left displaced abomasum (LDA) and the LDA was ruled out by Liptak test in these two animals. The Liptak test involved the determination of the pH of the fluid collected from the site of maximal ping sound intensity. Alkaline pH ruled out the LDA.

Table 2: Clinical examination results of 32 bovines with primary type 3 abomasal ulcer

Characteristic	Finding	Number of animals
General condition	Alert	07
	Moderately disturbed	25
Visual examination	No abnormality	12
	Mild left side distension	04
	Moderate left side distension	07
	Severe left side distension	01
	Bilateral distension	08
Mucous membrane	Normal	07
	Congested	14
	Anaemic	11
Dehydration	Not appreciable	03
	Mild	05
	Moderate	19
	Severe	05
Temperature	Low (<37.2°C)	01
	Normal (37.2-38.9°C)	20
	Increased (>38.9°C)	11
Heart rate/minute	Normal (60-80)	09
	Low (<60)	02
	Slightly increased (81-90)	07
	Moderately increased (91-100)	07
	Severely increased (>100)	07
Respiration rate/minute	Normal (15-25)	09
	Slightly increased (>25-35)	13
	Moderately increased (36-45)	08
	Severely increased (>45)	2
Rumen motility/2 minutes	Normal (3)	01
	Reduced (1)	06
	Reduced (2)	05
	Absent	19
	Increased (5)	01
Rumen consistency	Doughy	06
	Mushy	25
	Hard	1

Table 3: Rectal exploration findings in 32 bovines with primary type 3 abomasal ulcer

Characteristic	Finding	Number of animals
Rumen size	Normal	14
	Moderately distended	13
	Decreased	05
Intestines	Normal	16
	Distended	16
Rectal mucosa	Normal	15
	Sticky	17
Hindered hand movements	No	18
	Yes	14
Fecal quantity in rectum	Normal	10
	Reduced	01
	Scanty	15
	Negligible	04
	Absent	02
Fecal colour*	Normal	01
	Black	29
Fecal consistency	Normal	08
	Feces absent	02
	Pelleted	02
	Hard	01
	Loose	01
	Pasty	18
Presence of mucous	Absent	27
	Mucus with feces	04
	Mucus only	01

* Feces were present in rectum of 30 animals only

Laboratory findings

The significantly different hematological parameters between AU3 and control groups are presented in Table 4. The WBC was <4000/µL in two animals, 4000-8000/µL in six, 8001-12000/µL in eight, 12001-16000/µL in seven, and >16000/µL in nine animals. The toxic changes in neutrophils were observed in 15 cases and left shift in 24 cases. The hematological analysis was repeated only in 11 cases at 5-7 days post treatment. As the animals were at the owners premises, the animals were not examined at this time. The owners of animals that responded well to treatment typically showed up. The post treatment hematological analysis in comparison with pretreatment values revealed that there was a non-significant decrease in hemoglobin (9.98±0.73 to 9.84±0.32), WBC (13252±1827 to 11281±1046), and neutrophil count (7966±1191 to 6035±584); and a non-significant increase in lymphocyte count (5273±977 to 5615±811).

Table 4: Haematological and biochemical analyses of blood from 32 bovines with primary type 3 abomasal ulcer (mean±SEM)

Measurement	AU3	Control group
Haemoglobin (g/dL)	10.64±0.47*	11.79±0.21
Hematocrit (%)	28.4±2.5*	35.48±0.69
Platelet count ($\times10^3$)	347.3±48.9*	458.4±27.7
Erythrocyte count ($\times10^6$)	5.75±0.65	6.82±0.22
WBC (/µL)	13100±1362*	10100±295.3
Neutrophil (/µL)	8606±1241*	3637±124
Lymphocyte (/µL)	4477±422*	6242±195
BHBA (mmol/L)	2.97±0.11*	0.49±0.05
NEFA (mmol/L)	2.32±0.09*	0.30±0.03
Cholesterol (mg/dL)	63.9±6.1*	112.05±4.18
Triglyceride (mg/dL)	40.54±5.3	31.65±2.95
Glucose (mg/dL)	90.7±8.7*	60.45±1.75
Total bilirubin (mg/dL)	1.2±0.17*	0.14±0.02
AST (U/L)	286.6±41.7*	95.3±5.79
ALP (U/L)	101.6±14.9	83.45±4.67
GGT (U/L)	54.2±8.7	38.65±1.95
CK (U/L)	315.5±35.3*	69.55±6.45
LDH (U/L)	1857.7±119*	806.75±23.90
Total protein (g/dL)	6.58±0.24*	7.65±0.09
Albumin (g/dL)	2.73±0.13*	3.42±0.05
Globulin (g/dL)	3.84±0.22	4.23±0.05
Fibrinogen (mg/dL)	447.8±31.6	498.3±27.3
BUN (mg/dL)	34.1±3.1*	18.7±0.95
Creatinine (mg/dL)	2.27±0.23*	1.19±0.11
Lactate (mmol/L)	7.38±0.65*	1.44±0.14
Sodium (mmol/L)	136±1.95*	143.95±1.54
Potassium (mmol/L)	3.56±0.24*	4.54±0.10
Chloride (mmol/L)	90.7±2.4*	101.45±1.28
Calcium (mg/dL)	7.75±0.3*	9.48±0.23
Phosphorus (mg/dL)	5.3±0.31	5.9±0.11
Magnesium (mg/dL)	2.89±0.31	2.34±0.12

* Differ significantly between two groups at P≤0.05, t-test

The collected peritoneal fluid ranged from 2 ml to >3 L, but no fluid could be collected in two cases. The fluid had foul smell in eight cases. The peritoneal fluid characteristics are presented in Table 5. The common abnormal findings on the cytological examination of peritoneal fluid included neutrophilia, degenerated neutrophils, the aggregate of macrophages, fibroblasts, and mesothelial cells. Massive neutrophilia (neutrophils

>5000), and markedly degenerated neutrophils with engulfed bacteria along with the high count of activated macrophages and mesothelial cells were observed in three cases, while overwhelming peritonitis in five cases. The microscopic examination of peritoneal fluid smears showed the presence of bacteria in 70% of cases. These bacteria were cocci in nine samples (30%), bacilli in two (7%), and both cocci and bacilli in ten (33%). The bacteria were seen as free or phagocytized by the neutrophils. The presence of plant fiber (gut contents) in the peritoneal fluid was a consistent finding.

Table 5: Peritoneal fluid analysis results of 30 bovines with primary type 3 abomasal ulcer

Characteristic	Finding	Number of animals
Color	Crystal clear	04
	Yellow	22
	Reddish	03
	Pus like	01
Consistency	Watery	16
	Turbid	14
Volume	Up to 5 ml	07
	6-15 ml	05
	>15 ml	18
Specific gravity	1.005-1.015	04
	1.016-1.025	09
	1.026-1.038	17
WBC (/µL)	<3000	06
	3001-5000	16
	5300-7000	05
	8000-15000	03
Neutrophils (/µL)	<1800	02
	1800-3000	08
	3001-5000	16
	>5000	04
Neutrophils (%)	70-80	13
	81-90	09
	81-98	08
Total protein (g/dL)	<3.0	05
	3.1-4.5	17
	4.6-6.3	08
Bacteria	Absent	09
	Present	21

Imaging and other findings

All animals were negative for foreign body syndrome based on the findings of radiography and ferroscopy. Ultrasonography revealed fibrinous peritonitis with scanty effusion in the majority of animals, dilated abomasum (n=8), increased fluid in the peri-reticular area (n=4), and massive abscess in the right post xiphoid area (n=1). The mean rumen pH was 6.82±0.1 (median=7). Rumen chloride was normal (10-25 mmol/L) in six animals, and increased in 26 animals (mean=37.3±2.1, median=36.7 mmol/L). We were not able to collect abomasal fluid from two animals. The abomasal pH was 2-4 in 16 and 5-6 in 14 animals (mean=4.34±0.30, median=4). In the majority of animals, abomasal fluid was olive green in color. On sedimentation, sand settled in 18 samples of abomasal fluid.

Treatment

Twenty nine animals were treated with antibiotics, antacids, and ancillary therapy (Table 6). The right post xiphoid abscess in one animal was drained by free hand centesis. One animal (case No. 11) had been treated by the owner for 15 days, but the animal did not recover completely and was culled after 45 days. Eight non-survivors underwent postmortem examination. All had AU3, seven had type 1 ulcers, and the abomasa of five cases contained sand. All the survivors were healthy at the time of the last feedback. The pregnant animals had calved normally. Some of the nonpregnant animals had become pregnant, and then calved normally during the course of follow up. After recovery, the milk yield did not return to the previous levels in the current lactation, but there was no significant effect on the milk yield in the subsequent lactation.

Case No. 8, 9, 10, 18, 21, and 27, responded to treatment in terms of increased appetite and fecal output, but melena did not subside after initial treatment of 6-8 days. Hence, the antacid treatment was continued with MgO_2. Case 22 showed complete recovery in terms of fecal colour and appetite after 9 days of treatment but then showed inappetence just after another four days. Hematological analysis after 6 days of the second episode of inappetence revealed neutrophilic leukocytosis with mild left shift. The animal was then treated with antibiotics and MgO_2 for seven days and then with MgO_2 for another five days. We could not conclude whether it was the recurrence of the disease or the animal had not recovered completely.

Prognostic indicators

The statistical comparison was made between 16 survivors and 15 nonsurvivors. The final outcome of one animal was not known. The age, duration of illness, clinical signs, and clinical examination parameters did not differ significantly between the survivors and nonsurvivors. The left shift was observed in a higher percentage of nonsurvivors than survivors. The quantitative parameters which differ significantly between the survivors and nonsurvivors are presented in Table 7.

The physical characteristics and quantitative parameters of peritoneal fluid did not differ significantly between survivors and nonsurvivors. Septic peritonitis was observed in the higher percentage of nonsurvivors than survivors. The nonsurvivors had a greater degree of degenerative changes in neutrophils of peritoneal fluid than the survivors. None of the bovines with overwhelming peritonitis survived. Out of eight animals with foul smelling peritoneal fluid, only two survived.

Table 6: Treatment protocol for 29 bovines with primary type 3 abomasal ulcer

Case No.	Duration of illness	Antibiotics used	Antacid	Outcome	Outcome period
1	5	CAM	Ranitidine	Died	2
2	9	CAM	Ranitidine	Died	3
3	7	CAM	Ranitidine	Died	3
4	20	CAM	Ranitidine	Died	4
5	12	CAM	MgO_2	Died	5
6	12	CAM	MgO_2	Died	6
7	2	CAM	MgO_2	Died	6
8	8	CAM	Ranitidine	Recovered	10
9	5	CAM	Ranitidine	Recovered	10
10	6	CAM	Ranitidine	Recovered	30
11	12	CAM	Ranitidine	Culled	45
12	8	AEM	Ranitidine	Died	2
13	10	AEM	Ranitidine	Died	4
14	15	AEM	Ranitidine	Died	6
15	7	AEM	MgO_2	Recovered	7
16	30	AEM	Ranitidine	Recovered	9
17	30	AEM	MgO_2	Recovered	23
18	3	AEM	Ranitidine	Recovered	10
19	15	AGM	Ranitidine	Died	4
20	9	AGM	Ranitidine	Recovered	10
21	15	AGM	Ranitidine	Recovered	12
22	8	AGM	MgO_2	Recovered	30
23	3	AG	MgO_2	Recovered	5
24	15	AG	MgO_2	Recovered	8
25	7	AG	MgO_2	Recovered	11
26	6	SGM	Ranitidine	Died	3
27	7	SGM	Ranitidine	Recovered	10
28	4	SG	Ranitidine	Recovered	7
29	20	SG	MgO_2	Recovered	26

In column 3, C: Ceftiofur, A: Ampicillin, M: Metronidazole, E: Enrofloxacin, G: Gentamicin, and S: Streptopencillin

Table 7: Statistically different parameters between survivors and non-survivors of primary type 3 abomasal ulcer in bovines (mean±SEM)

Parameter	Survivors (16)	Non-Survivors (15)
Total bilirubin (mg/dL)	0.79±0.12	1.47±0.31*
CK (U/L)	216.7±24.5	411.87±60.9*
LDH (U/L)	1421.6±101.5	2350.1±150.6*
Total protein (g/dL)	7.39±0.3	5.72±0.274*
Albumin (g/dL)	3.06±0.18	2.34±0.14*
Globulin (g/dL)	4.33±0.35	3.38±0.23*
BUN (mg/dL)	27.56±3.94	38.47±3.93*
Creatinine (mg/dL)	1.53±0.14	2.85±0.35*
Rumen chloride (mmol/L)	31.23±2.32	43.0±2.88*

* Differ significantly between two groups at $P \leq 0.05$, t-test

Discussion

The AU3 was diagnosed on the basis of clinical evaluation, abnormal abdominocentesis findings, and ultrasonography or necropsy after ruling out TRP (Constable, 2010). Ultrasonography was helpful in ruling out other causes of localized peritonitis but did not aid in the localization and diagnosis of AU3. The majority of animals in our study had melena, hence type 2 abomasal ulcer and intussusception were also ruled out (Hussain *et al*., 2015b; Braun *et al*., 2019). Further, the absence of clinical signs of anemia ruled out the possibility of type 2 abomasal ulcer but not of type 1 abomasal ulcer. Aspiration of blood or hemorrhagic fluid during abomasocentesis is highly suggestive of bleeding abomasal ulcers (Braun *et al*., 1997; Braun, 2009). In this study, the absence of hemorrhagic abomasal fluid on abomasocentesis ruled out the possibility of type 2 ulcers. Melena was attributed to type 1 abomasal ulcers, and such ulcers were also observed on necropsy. The possibility that type 1 ulcer may have led to perforation could not be ruled out. The presence of AU3 on the postmortem of eight animals suggested that the clinical diagnosis was correct.

The occult blood by HEMOSPOT test cards revealed the presence of melena in AU3 buffaloes. Likewise, in a recent study, Yasaswini *et al*. (2021) used the benzidine test as a fecal occult blood test to confirm the abomasal ulcers in buffaloes. Nevertheless, there exists a scope of uncertainties in methodology because of the sensitivity differences of the fecal occult blood test, which has to be taken into consideration (Munch *et al*., 2020).

Most cases of AU3 have been reported within one month of parturition (Palmer and Whitlock, 1984; Braun *et al*., 2019) but 43% of cases in one study occurred during late lactation (Cable *et al*., 1998). Smith *et al*. (1983) observed no relationship between the early postpartum or peak lactation periods and the ulcer prevalence. In all these above quoted studies, majority (86-100%) of the affected animals had one or more concurrent diseases (abomasal displacement, metritis, mastitis), which mostly occur during the immediate postpartum period. This finding that AU3 are found especially in the early postpartum period, and are

therefore presumably associated with the stress of calving was not supported by our data. Although recent calving did not seem to be the predisposing factor, the stress of high milk production cannot be ruled out. In pregnant animals, the stress of pregnancy could be a possible predisposing factor. Another factor may be decreased abomasal perfusion caused by the shunting of a higher percentage of cardiac output to the fetus or lactating udder (Fubini *et al*., 2018). However, no adequate studies prove that there is relative hypoperfusion on the loci, predisposed to the development of ulcers. There is a possibility that irritation of the abomasal wall by the sand could have initiated the abomasal ulcers.

The clinical findings resembled TRP and were similar to recently published findings (Braun *et al*., 2019), except for a higher percentage of melena in the current study. Similar to our results, Smith *et al*. (1986) observed melena in 80% of AU3 cases. The site of post xiphoid pain localization may help to differentiate AU3 and TRP. With TRP, the pain would be maximal on the left, and with AU3, the pain would be maximal on the right (Palmer and Whitlock, 1984). Similar to the present findings, Smith *et al*. (1986) observed pain on palpation of cranio-ventral abdomen region in 54% (14/26) of cattle suffering from abomasal ulceration. A few case reports have also documented pain on palpation of right post xiphoid area in AU3 (Costa *et al*., 2002; Hussain *et al*., 2011).

The percentage of animals with colic in our study was similar to earlier studies that reported abdominal pain in 45-80% of animals with AU3 (Smith *et al*., 1986; Braun *et al*., 2019). Pneumo-peritonium not associated with surgery has been reported to be indicative of bacterial peritonitis and presence of gas producing bacteria (Constable *et al*., 2017). All the animals with pneumo-peritonium were having septic peritonitis on peritoneal fluid examination. The absent or reduced rumen motility may be attributed to peritoneal inflammation, toxemia, and hypocalcemia. Mushy rumen consistency may be attributed to prolonged retention of ingesta in rumen due to ineffective or absent rumen contractions. It is important to mention that in some cases previous treatment had resulted in mild improvement in appetite in few cases, but clinical recovery was not complete.

The hematological alterations ranged from marked neutrophilia to leukopenia with left shift and presence of toxic neutrophils. None of the animals had normal hemogram. In conformity with our results, Tharwat and Ahmed (2012) observed neutrophilia in bovine abomasal ulceration. Braun *et al*. (2019) observed normal WBC (5000-10000) in 63% of cows with secondary AU3. Differential cell count was not reported in that study; hence, it is difficult to interpret whether those 63% of cows had a normal leukogram or not. Although the repeated hematological analysis of survivors did not reveal significant changes in comparison to pretreatment analysis, there was a decrease in WBC and neutrophil counts, indicating a positive response to therapy.

The increased levels of liver enzymes indicated impaired hepatic function and/or hepato-biliary circulation. The increased levels of BHBA and NEFA confirmed that animals with AU3 suffered from disturbance in glucose and lipid metabolism (Li *et al*., 2016). Increased BHBA and NEFA indicated ketosis but it was considered as secondary ketosis due to prolonged anorexia and not a primary disease. There could be two possible reasons for lower total protein and albumin. Firstly, third space losses followed by reduced synthesis of albumin (negative acute phase protein) by the liver. Hemoconcentration, yet a tendency for normal total protein suggested significant third space losses (Hussain *et al*., 2015b).

The probable causes of hyperlactatemia in the present study could be dehydration, reduced hepatic utilization, and decreased renal clearance due to azotemia. Abomasal reflux could be the cause of dehydration, hypochloremia, hypokalemia, and azotemia (Constable *et al*., 2017; Hussain *et al*., 2021a). Third space losses may have resulted in low sodium and hypocalcemia was ascribed to decreased absorption and anorexia (Constable *et al*., 2017).

An increased number of peritoneal fluid leukocytes is commonly associated with inflammation (Kopcha and Schultze, 1991; Fubini *et al*., 2018), but the sensitivity and specificity of this parameter is low. Peritoneal fluid nucleated cell count of greater than 6000/µL has been reported to be consistent with the diagnosis of peritonitis in cattle (Hirsch and Townsend, 1982; Ward and Ducharme, 1994). The nucleated cell count criterion did not hold well in the present study, rather, differential cell count of peritoneal fluid was more useful in the diagnosis of peritonitis as WBC was <6000 in 78% of the cases. The consistent findings on peritoneal fluid examination in the present study were increased specific gravity and >50% neutrophils. A total protein concentration of >3 g/dL in the peritoneal fluid was observed in most cases and this concentration has typically been used as the cut-off value for exudates (Kopcha and Schultze, 1991; Constable *et al*., 2017).

The common cytological abnormalities of peritoneal fluid were an increased number of neutrophils with degenerative or toxic changes. The presence of gut contents in the peritoneal fluid was ascribed to the perforated abomasal ulcer. Overwhelming peritonitis was characterized by a massive number of bacteria, greater than the cells in peritoneal fluid with markedly degenerated neutrophils. This severe peritonitis was indicative of a poor prognosis.

According to Smith *et al*. (1983), abomasal ulceration management requires antibiotics, antacids, and dietary changes. Clinical trials for determining different drug concentrations in abomasal ulceration induced peritonitis are not available (Constable *et al*., 2017). Third generation cephalosporin or synthetic penicillins have been reported to be good choices in peritonitis (Fubini *et al*., 2018). Hence, both were used in the present study. The antibiotic combination did not seem to affect the final outcome. As per the protocol of our clinic, the

preferred antibiotic combination for bovines with severe hematological and peritoneal fluid alterations was ceftiofur, ampicillin, and metronidazole, but 64% of animals treated with this combination did not recover. The reason for unresponsiveness in these animals may be severe peritonitis, and toxemia leading to the inhibitory effect on gastrointestinal motility. The animals that recovered were having less degree of degenerative changes in the peritoneal fluid. The survival rate was 55.2% (n=16/29). The use of corticosteroids and non-steroidal anti-inflammatory drugs could have been beneficial but were avoided because of their ulcerogenic property.

Although the clinical effect of oral antacids has not been evaluated in adult cattle and buffaloes, the recommended antacids for adult ruminants are magnesium oxide or aluminum hydroxide (Palmer and Whitlock, 1983; Constable *et al*., 2017). To the valuable cattle or calves, intravenous histamine type 2 blockers can be administered (1.5 mg/kg ranitidine every 8 h) (Ahmed *et al*., 2001; Fubini *et al*., 2018). In the present study, both parental and oral antacids were used as adjuvant therapy for AU3. Despite the higher percentage of recovery rate in MgO_2 cases compared to ranitidine treated cases, the difference was not statistically significant. Whether supportive therapy had or had not any effect on the improvement of the animals could not be concluded. The recovery rate was (55.2%) similar to an earlier study (Tharwat and Ahmed, 2012) but slightly lower than the study of Braun *et al*. (2019). A surgical procedure for AU3 was not attempted because of the presumable complication of abomasal rupture or even diaphragmatic hernia while attempting to break the adhesions (Cable *et al*., 1998).

Higher derangement of liver and kidney functions, higher rumen chloride, the presence of left shift, and low total protein, albumin, and globulin were associated with lower survival rates. In nonsurvivors, the severity of infection could have increased with time, resulting in death. It is always advisable to take multiple samples at different time intervals or stages of the disease to predict the exact prognosis (Hussain *et al*., 2021a). There is possibility of recurrence of AU3 (Braun *et al*., 2019); however, in this study, no recurrence was recorded during the follow-up period.

Primary AU3 can occur during any stage of lactation and in pregnant animals as well. The clinical features of primary AU3 resembled TRP except for melena. The hematological analysis revealed low hemoglobin, hematocrit, and platelet count with neutrophilia. Biochemical alterations revealed moderate or severe hepatic, and renal malfunction, negative energy balance, hypocalcemia, hyponatremia, hypokalemia, hypo-chloremia, and hyperlactatemia. In treatment, the deficient electrolytes and minerals and negative energy balance should be corrected in addition to the use of antibiotics and liver tonics. The response to medical treatment was fair. The long-term survival rate was good, and there was no recurrence. Septic peritonitis, left shift, higher derangement of liver function and kidney function, high rumen chloride, and low total protein, albumin, and globulin were the negative prognostic signs.

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Conflict of interest

The authors declare that there is no conflict of interest.

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