Benha Veterinary Medical Journal 39 (2020) 59-67

Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Ultrasonographic, clinical and haemato-biochemical evaluations of rumino-cutaneous fistula in buffaloes (*Bubalus bubalis*)

Ahmed H. Khalil[1,*], Mohamed M. Zeineldin[2] and Mona A. El Shemy[3]

[1] *Department of Veterinary Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, Benha University, Egypt.*

[2] *Department of Animal Medicine, Faculty of Veterinary Medicine, Benha University, Egypt.*

[3] *Department of Clinical Pathology, Faculty of Veterinary Medicine, Benha University, Egypt*

ARTICLE INFO

Keywords

Buffalo
Clinical
Hematobiochemical
Ruminal fistula
Ultrasonography

Received 24/09/2020
Accepted 25/10/2020
Available On-Line 20/01/2021

ABSTRACT

One of the most prevalent postoperative complications following rumenotomy is rumino-cutaneous fistulation, which can cause significant economic issue in ruminant. The aim of this study was to determine the clinical, hematobiochemical, and ultrasonographic variations in rumino-cutaneous fistulation before and after surgical correction in buffaloes. Five buffaloes with rumino-cutaneous fistulation and five clinically healthy buffaloes were evaluated in this study. Buffaloes with rumino-cutaneous fistulation exhibited reduced appetite, decreased rumen motility, scanty feces, and significant alterations in hematobiochemical parameters compared with clinically healthy control buffaloes. Ultrasonographically, buffaloes with rumino-cutaneous fistulation displayed a significantly lower rate of rumen contractions and a substantial increase in the thickness of ruminal and abdominal walls. Significant postoperative improvement in clinical, hematobiochemical and ultrasonographic parameters in affected buffaloes was noted. In conclusion, rumino-cutaneous fistula is a common surgical complication of rumenotomy and rumen trocarization in buffaloes. Surgical interference by excision of fistulous tract is satisfied for treatment. Ultrasonographic, clinical and hematobiochemical examinations are essential for the proper assessment of rumino-cutaneous fistulation before and after surgical correction in buffaloes.

1. INTRODUCTION

Rumenotomy is a safe, common, and efficient surgical procedure for the correction of ruminal tympany, ruminal impaction, and traumatic reticuloperitonitis in ruminants (Dehghani and Ghadrdani, 1995; Niehaus, 2008). However, it has been associated with many postoperative complications, such as local to extensive diffuse proliferative inflammatory adhesions, peritoneal abscess, and tissue hyperplasia (William *et al.*, 1990; Hartnack*et al.*, 2015). Rumenotomy can also result in rumino-cutaneous fistulation with discharge of ruminal juice at the operation site, significant drop in milk production, anorexia, and persistent fever (William *et al.*, 1990; Devi Prasad *et al.*, 2014). Rumino-cutaneous fistulation is an anomalous interaction between the rumen and skin that results in major economic losses in ruminants (Omid and Mozaffari, 2014; and Singh *et al*., 2016). Outflow of leaked high amounts of ruminal juice from rumino-cutaneous fistulation in buffaloes causes skin lesions, dehydration, malnutrition, unpleasant odor, myiasis and septic peritonitis (Rafee *et al.*, 2015). Moreover, the increase in the treatment costs and potential fatalities due to resultant septic peritonitis (Sileshi *et al.*, 2013).

The closure rates of rumino-cutaneous fistulation vary substantially depending on the degree of fistulation (Omid and Mozaffari, 2014). In addition, the correct description of rumino-cutaneous fistula anatomy is important for further planning of operative repair (Rafee *et al.*, 2015). Therefore, establishing and maintaining successful fistula recovery involves rapid surgical intervention with continuing reassessment of wound closure over time.

Difficulty in assessing the sequelae of rumino-cutaneous fistulation by physical examination has led to the adoption of innovative diagnostic tools for fistula assessment before and after surgery to minimize the economic burden on buffalo production. Ultrasonography has been suggested as a noninvasive technique for the diagnosis and assessment of much gastrointestinal affection and to describe fistula anatomy before and after treatment in ruminants (Saini *et al.*, 2007).

To best of our knowledge, there have been no studies regarding rumino-cutaneous fistula assessment in buffaloes. The aim of this study was to assess the ultrasonographic, clinical and hematobiochemical differences in rumino-cutaneous fistulation before and after surgical correction in buffaloes.

* Corresponding author: Ahmed Khalil, Faculty of Veterinary Medicine, Benha University, Benha, Egypt. Email: ahmed.khalil@fvtm.bu.edu.eg

2. MATERIAL AND METHODS

2.1. Ethics statement

The study procedures were conducted in compliance with the guidelines for the care and use of animals at the College of Veterinary Medicine, Benha University. The research protocol was approved by the Ethical Committee for Institutional Animal Use and Care of the College of Veterinary Medicine, Benha University (BUFVTM 15-09-2018).

2.2. Animals and study groups

Five buffaloes (*Bubalus bubalis*; one male and four females) aged between 1 and 8 years and weighing between 220 and 550 kg were included in this study. All cases were admitted to the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Benha University, Egypt. All buffaloes suffered from left flank fistulation discharging ruminal ingesta. Three cases had a history of rumenotomy intervention (since 3-5 weeks), and the other two cases had undergone numerous rumen trocharization (since 4-7 weeks) attempts to resolve persistent tympany. All buffaloes underwent surgery to excise the fistulous tract and to break the internal adhesion. In addition, we selected five apparently healthy buffaloes (1–8 years old and 220-550 kg weight) from the educational ruminant farm at the College of Veterinary Medicine, Benha University, and used as a control group. Based on the clinical evaluation of heart rate, respiratory rate, ruminal movement, and rectal temperature, all selected control buffaloes were determined to be clinically healthy.

2.*3. Timeline and evaluation of surgical interference*

The procedures for the surgical excision of the fistulous tract and closure of the rumen and abdominal wall layers were evaluated before surgery on day 0 and postoperative on days 3, 10, 20, and 30. Evaluation consisted of clinical, hematobiochemical, and ultrasonography examinations.

2.4. Clinical evaluation

Clinical evaluation of heart rate, respiratory rate, ruminal movement, and rectal temperature were performed in all affected and clinically healthy buffaloes. In addition, fistula probing and pinching of withers to evaluate the pain test were performed as described by Abdisa (2017).

2.5. Ultrasonographic evaluation

After hair clipping and application of ultrasound coupling gel, transcutaneous ultrasonography was performed in standing position. All ultrasonographic evaluations were performed using a portable ultrasound machine (Chison ECO3-Expert, Medical-EXPO, China) with an adapted 5 MHz convex probe. On the left side, the rumen and peritoneum were examined in a consistent manner from the eighth intercostal space to the flank (Abouelnasr et al., 2012). The assessed ultrasonographic ruminal feature included; the rate of ruminal contractions (per 3 minutes), period of rumen relaxation, period of ruminal contraction, thickness of ruminal wall and thickness of the abdominal wall.

2.*6. Hematological and biochemical examination*

Blood samples were collected from the jugular veins previously described by (Abd Ellah et al., 2014).Blood samples were collected from both clinically healthy buffaloes and affected buffaloes at baseline (day 0) and then at days 3, 10, 20, and 30 postoperative. Blood samples were collected into three tubes, the first tube on EDTA for hematological examination, the second tube is plain centrifuge tube for serum collection for biochemical analysis [total proteins, albumin, aspartate amino transferase (AST), alanine amino transferase (ALT), blood urea nitrogen (BUN), creatinine, sodium, potassium, malondialdehyde (MDA) and tumor necrosis factor alpha(TNF-α)] and the third heparinized tube for collection of plasma. Blood plasma and serum samples were collected after centrifugation and then kept frozen at -20 °C until required. Heparinized tubes were kept frozen for measuring of acute phase proteins such as fibrinogen and C Reactive Protein (CRP).

A complete blood count (erythrogram and leukogram) was determined through the hematology analyzer (Medonic CA 620). Plasma fibrinogen was measured by using commercially bovine kits, according to the method described by (Orro *et al.*, 2011). Serum total proteins, albumin, urea nitrogen, Creatinine, AST and ALT were examined by commercial kits from Diamond Diagnostics, Egypt according to the instruction of manufacturer.

Serum sodium and potassium levels were estimated by using kits from Spectrum Diagnostic according to the instruction of manufacture.

Serum MDA level was measured and expressed in nmol/ml of serum according to Pertile et al., (1995). Serum TNF- α and plasma CRP were estimated by using kits from Kamiya Biomedical Company according to the instruction of manufacture.

2.7. Surgical interference

The affected buffaloes were restrained in a standing position, and the surgical site was aseptically cleaned immediately before initiation of the surgical procedure. Surgical intervention was done after sedation with xylazine HCl (Xylaject®, ADWIA-Comp., Egypt), administered intramuscularly at a dose of 0.1 mg/kg body weight and inverted- L local analgesia using 60–80 mL of 2% lignocaine hydrochloride (Depocain®, Arab Co., A.R.E.). Elliptical incisions of 15-20-cm long and 5-10-cm wide including the fistulous tract and the enclosed damaged tissue were performed through the skin, muscular layers of the abdominal wall, and the underlying peritoneum and rumen. All layers were identified and sharply dissected from each other, and the peritoneal cavity was investigated to detect any adhesion that was bluntly dissected. The rumen was fixed with four stray stitches at the dorsal, ventral and both

sides to be handpicked with assistants. Rumen, reticulum, and reticulo-omasal orifices were examined for any foreign body or over contents. The ruminal wall was sutured with a double line of continuous Cushing sutures using XLG, No. 2 (Vicryl, Ethicon-Ltd., UK). The peritoneum with the transverse abdominis muscle and the external with the internal abdominal oblique muscles were sutured together by simple continuous pattern using the same suture material. Subcutaneous tissue was sutured in the same manner; the skin was sutured using a ford interlocking pattern with silk (Ethicon-Ltd., UK) No. 3. The surgical procedures used for rumino-cutaneous fistula correction in the buffaloes are depicted in (Figure 1A–E).

2.8. Postoperative care

Penicillin-streptomycin (20000 IU/kg – 20 mg/kg, IM) and Flunixin meglumine (2.2 mg/kg; Flamicure, Pharma-Swede, 10th of Ramadan-City, Egypt) were injected for five days postoperative, and the wound was daily dressed using Betadine antiseptic solution.

2.9. Statistical analysis

Statistical analysis was carried out using the statistical software package SPSS for Windows (Version 16.0; SPSS Inc., Chicago, Ill). The significance of differences between more than two groups was estimated by one-way ANOVA. If there were significant differences between individual groups by one-way ANOVA, these differences were estimated by LSD test. Results were presented as the mean ± SEM. *P*-value of less than 0.05 was considered significant

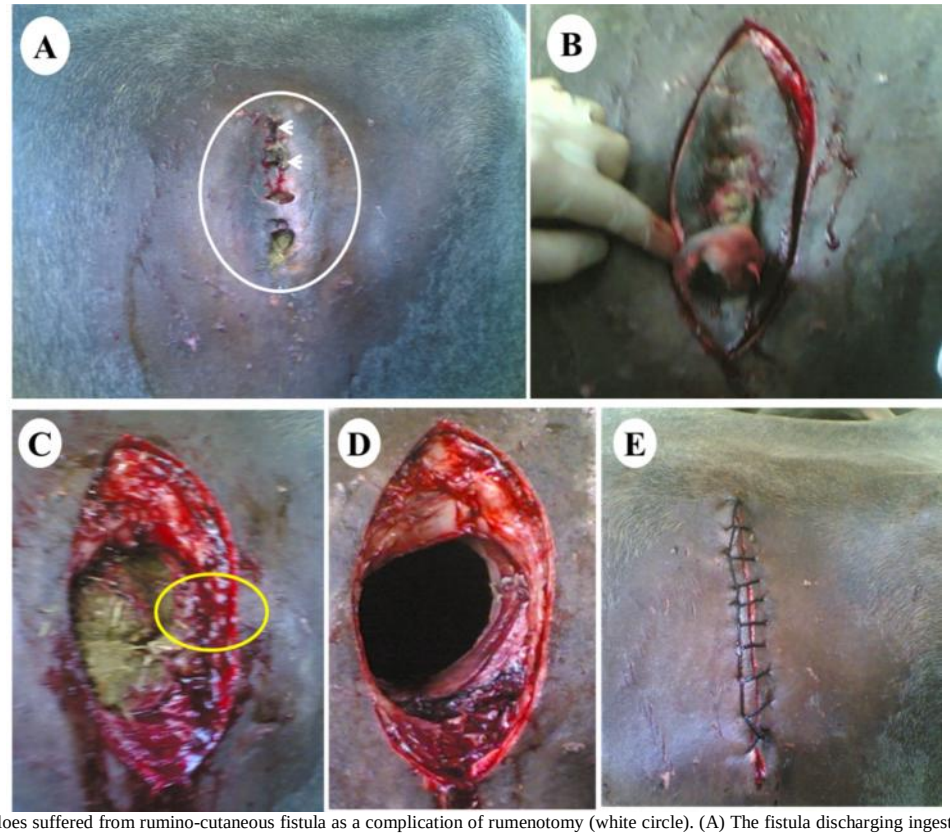

Figure 1 A buffaloes suffered from rumino-cutaneous fistula as a complication of rumenotomy (white circle). (A) The fistula discharging ingesta with a remnant of nonabsorbable suture material (silk) at the fistulous tract opening (arrow heads) . (B) Elliptical incision for excision of fistulous tract and surrounded thickened layers. (C) Fully distended rumen and thicken adhered skin, muscle layers, and ruminal serosa (yellow circle). (D) Abdominal wall layers after dissection. (E) Skin wound after suturing by Ford interlock layer.

3. RESULTS

3.1. Clinical examination

Clinical findings, history, and owners' complaints of buffaloes with rumino-cutaneous fistula were summarized in (Table1). All admitted buffaloes suffered from left flank fistulation and discharging ruminal ingesta with or without pus. Buffaloes were suffered from decreased appetite (5/5), recurrent tympany (3/5), ruminal atony (5/5), scanty hard stool (4/5), scanty soft mucoid feces (1/5), systemic reactions (3/5), pain (2/5) and regurgitation of food (1/5). Intraoperative examination showed accumulation of undigested materials such as plastic bags (3/5), wire and nails (1/5), and frothy contents (2/5). Naked eye inspection at the fistula site in cases due to several trocharization attempts, revealed an evidence of several trocarization points with stunted ruminal papillae in 2 animals (Figure 2A).

Clinical parameters (rectal temperature, respiratory rate, and heart rate) in both clinically healthy and affected buffaloes were listed in Table (2). Affected buffaloes showed a significant ($P \leq 0.05$) increase in rectal temperature, respiratory rate, and heart rate compared with clinically healthy buffaloes. Postoperatively, rectal temperature, respiratory rate, and heart rates showed non-significant ($P \leq 0.05$) changes on days 20, 10, and 30 compared with clinically healthy buffaloes (Table 2).

3.2. Hematological and biochemical examination

As shown in Table-3a, in comparison with the normal group, buffaloes of the affected group at the 3rd postoperative day revealed a significant decrease in RBCs count, hemoglobin content, HCT%, serum (total proteins, albumin, sodium and potassium level) with a significant increase in TLC, MCV, plasma fibrinogen, serum (AST, ALT, BUN and Creatinine). Meanwhile, there was non-significant change in MCHC.

At the 10thpostoperative day, there was a significant decrease in RBCs count, HCT%, serum (total proteins, sodium and potassium) with a significant increase in TLC, serum (AST and ALT) meanwhile, the Hb, MCV, MCHC, plasma fibrinogen, BUN, serum Creatinine and albumin showed non-significant changes in the affected group when compared to the normal group (Table-3a).

At the 20th postoperative day, there was a significant decrease in HCT%, serum (total proteins, sodium and potassium) with a significant increase in serum ALT and plasma fibrinogen. Meanwhile, there were non-significant changes in RBCs count, Hb, MCV, MCHC, TLC, serum (albumin, AST, BUN and Creatinine) in the affected group when compared to the normal group (Table-3a).

At the 30th postoperative day, the affected group showed non-significant changes in RBCs count, Hb, HCT%, MCV, MCHC, TLC, plasma fibrinogen, serum (total proteins, albumin, ALT, BUN, sodium and potassium)with a significant decrease in serum (AST and Creatinine) when compared to normal group (Table-3a).

Animals before surgical operation showed significant decrease in RBCs count, Hb, HCT%, serum (total proteins, albumin, sodium and potassium) with significant increase in MCV, TLC, MCHC, plasma fibrinogen, serum (ALT, AST, BUN and Creatinine) when compared with normal animals.

In comparison with the animals before surgical operation, animals at the 30th postoperative day showed significant increase in RBCs count, Hb, HCT%, serum (total proteins, albumin, sodium and potassium) with significant decrease in MCV, TLC, plasma fibrinogen, serum (AST, ALT, BUN, BUN, Creatinine, sodium and potassium).

Serum MDA level and TNF-α showed significant increase in the animals before the surgical operation and in animals at the 3rd, 10th and 20th postoperative day when compared with normal animals and animals at the 30th postoperative day. Meanwhile, there was non-significant change in MDA level and TNF-α in the group at the 30th postoperative day when compared with normal animals (Table-3b).

Plasma CRP showed significant increase in the animals before the surgical operation and in animals at the 3rd, 10th, 20th and 30th postoperative day when compared with normal animals. In comparison with animals at the 30th postoperative day, animals before surgical operation revealed significant elevation in the plasma CRP (Table-3b).

3.3. Ultrasonographic examination

Ultrasonographic examination of rumen in the clinically healthy buffaloes showed a thin echogenic wall lining with an anechoic shadow underneath due to rumen gaseous contents (Figure 2B). The fistulous tract in the affected buffaloes displayed a hyperechoic band with prominent acoustic shadowing resulting from the ultrasound pulse reflection from the fibrous connective tissue (Figure 2C). Ultrasonographic examination of the surrounded rumen wall of the affected buffaloes showed thicker echogenic particles of varying degrees of frequency punctuated by hypoechogenic fluid patches (Figure 2C). Therefore, it was difficult to differentiate between the different layers of ruminal wall. Ruminal wall abscess was reported in two buffaloes (Figure 2D and 2E) and appeared as anechoic to hypoechoic wall cavitation surrounded by an echogenic capsule with a diameter ranging from 0.4–0.8 cm (Figure 2F).

Table 1 History and clinical findings in 5 buffaloes with rumino cutaneous fistula.

Items	Buffalo-1	Buffalo-2	Buffalo-3	Buffalo-4	Buffalo-5
Age	1 y	1 y	4.5 y	6 y	8 y
Sex	Male	Female	Female	Female	Female
Previous interference	Trocarization (more than 10 times)	Rumenotomy (suturing all layers by non-absorbable suture material)	Trocarization (more than 10 times)	Rumenotomy (suturing the rumen with peritoneum and muscle layers)	Rumenotomy (suturing the rumen with peritoneum & muscle layers)
Reduced appetite	+	+	+	+	+
Ruminal atony	+	+	+	+	+
Recurrent tympani	+	-	+	+	-
Scanty hard feces	-	+	+	+	+
Scanty soft feces	+	-	-	-	-
Systemic reactions	+	-	+	+	-
Positive pain tests	+	-	+	-	-
Regurgitation of food	-	+	-	-	-
Local peritonitis	-	+	-	+	+
Frothy contents	+	-	+	-	-
Pus formation	+	-	+	+	-
Fistulous opening (s)	Multiple	Multiple	Multiple	Multiple	Single

Table 2 Vital parameter differences between healthy control group and affected group with rumino cutaneous fistula before and after surgical interference

Items	Normal group	Before	Postoperative			
			3rd d.	10th d.	20th d.	30th d.
Respiratory rate/minute	17.00±1.58^{c}	29.00±2.55^{b}	31.60±3.54^{a}	27.00±1.58^{c}	20.00±1.58^{d}	18.6±1.14^{de}
Pulse rate/minute	85.40±9.29^{b}	112.20±10.57^{a}	122.60±9.91^{a}	115.20±11.80^{a}	115.20±11.80^{a}	88.60±8.79^{b}
Rectal temperature (C°)	38.32±0.37^{c}	39.76±0.34^{a}	39.90±0.10^{a}	39.02±0.19^{b}	38.56±0.30^{c}	38.28±0.34^{c}

The mean within the same row having different superscripts (letters) are significantly different at level ($p \leq 0.05$)

Table 3a Haemato-biochemical parameters differences between healthy control group and affected group with rumino cutaneous fistula before and after surgical interference

Items	Normal group	Before	Postoperative			
			3rd day	10th day	20th day	30th day
RBCs 10^6/µL	6.18±0.37^{a}	4.32±0.45^{c}	4.20±0.49^{c}	5.20±0.49^{b}	5.92±0.34^{a}	5.96±0.57^{a}
Hb gm/dL	10.01±0.63^{c}	8.79±0.62^{ab}	8.49±0.51^{a}	9.33±0.57^{c}	9.44±0.61^{c}	10.00±0.61^{c}
HCT%	26.93±0.16^{a}	21.5±1.29^{d}	21.60±1.18^{d}	23.90±0.64^{c}	25.78±0.48^{b}	26.29±1.16^{ab}
MCVfL	43.70±1.23^{a}	57.66±1.17^{c}	51.91±2.16^{bc}	46.19±0.16^{a}	43.66±1.20^{a}	44.46±2.16^{a}
MCHC g/L	37.18±1.10^{a}	35.46±1.8^{b}	39.34±1.20^{ab}	39.05±1.01^{ab}	36.63±1.10^{ab}	38.04±1.10^{a}
TLC 10^3/µL	8.73±0.50^{c}	10.0±0.65^{b}	10.67±0.57^{a}	10.05±0.47^{ab}	9.28±0.42^{c}	8.90±0.58^{c}
Total Protein g/dL	5.9±2.08^{a}	5.05±3.45^{bc}	4.73±4.48^{d}	4.89±4.04^{cd}	5.38±4.07^{bc}	5.79±4.24^{a}
Albumin g/dL	3.37±2.64^{a}	2.89±3.09^{b}	2.81±2.84^{b}	3.05±2.39^{ab}	3.07±4.71^{ab}	3.29±2.73^{ab}
Fibrinogeng/l	4.22±0.35^{c}	8.14±0.37^{a}	8.09±0.61^{a}	7.35±1.27^{b}	5.73±0.43^{b}	4.45±2.28^{c}
AST U/L	70.47±1.27^{b}	76.49±2.50^{d}	77.24±1.72^{d}	73.55±1.87^{c}	71.55±0.69^{bc}	68.65±2.31^{a}
ALT U/L	26.81±0.96^{c}	32.90±1.84^{a}	30.96±4.96^{ab}	30.51±2.39^{ab}	29.51±1.48^{b}	26.01±2.76^{c}
BUN mmol/L	8.80±0.51^{b}	10.40±0.82^{a}	10.62±0.89^{a}	9.64±0.55^{ab}	8.64±0.55^{b}	8.80±1.04^{b}
Creatinine µmol/L	44.14±3.74^{bc}	50.49±3.42^{a}	48.65±4.82^{a}	42.85±2.56^{bc}	39.94±0.63^{cd}	38.34±2.32^{d}
Sodium mmol/L	131.60±2.07^{a}	107.00±5.20^{d}	105.20±7.29^{d}	113.8±9.39^{cd}	121.80±7.56^{bc}	126.60±5.86^{ab}
Potassium mmol/L	8.59±0.57^{a}	6.84±0.27^{d}	6.04±0.58^{d}	7.67±0.52^{cd}	7.94±0.58^{bc}	8.14±0.46^{ab}

The mean within the same row having different superscripts (letters) are significantly different at level ($p \leq 0.05$).

Table 3b MDA level, CRP and TNF-α difference between healthy control group and affected group with rumino cutaneous fistula before and after surgical interference

Items	Normal group	Before	Postoperative			
			3rd day	10th day	20th day	30th day
MDA (nmol/ml)	06.20±0.14^{a}	009.60±0.21^{d}	09.46±0.28^{d}	008.80±0.13^{c}	007.98±0.29^{b}	06.80±0.22^{a}
CRP (mg/L)	21.88±0.15^{a}	282.12±2.20^{e}	279.10±2.9^{e}	191.76±2.1^{d}	146.30±2.5^{c}	44.18±0.99^{b}
TNF- α (pg/ml)	11.10±0.27^{a}	022.50±0.69^{d}	19.80±0.65^{c}	19.32±0.32^{c}	13.86±0.48^{b}	12.20±0.20^{a}

The mean within the same row having different superscripts (letters) are significantly different at level ($p \leq 0.05$)

3.3. Ultrasonographic examination

Ultrasonographic examination of rumen in the clinically healthy buffaloes showed a thin echogenic wall lining with an anechoic shadow underneath due to rumen gaseous contents (Figure 2B). The fistulous tract in the affected buffaloes displayed a hyperechoic band with acoustic shadowing resulting from the ultrasound pulse reflection from the fibrous connective tissue (Figure 2C). Ultrasonographic examination of the surrounded rumen wall of the affected buffaloes showed thicker echogenic particles of varying degrees of frequency punctuated by hypoechogenic fluid patches (Figure 2C). Therefore, it was difficult to differentiate between the different layers of ruminal content. Ruminal wall abscess was reported in two buffaloes (Figure 2D and 4E) and appeared as anechoic to hypoechoic wall cavitation surrounded by an echogenic capsule with a diameter ranging from 0.4–0.8 cm (Figure 2F).

Ultrasonographic findings of ruminal features in affected and clinically healthy buffaloes were presented in Table (4). Rate of rumen contractions in the affected buffaloes were significantly decreased compared with clinically healthy buffaloes (1.20±0.45 vs 3.80±0.71, respectively). On the 30th postoperative day, the rates of rumen contractions in operated animals showed non-significant changes compared with the clinically healthy group.

In the affected buffaloes, the durations of rumen contractions (5.00±0.71 sec.) and relaxation (123.20 ± 9.34 sec.) were significantly longer than in clinically healthy buffaloes (2.40 ± 0.55 and 65.40 ± 8.26 sec., respectively). On the 30th postoperative day, the affected buffaloes displayed a non-significant change in the duration of rumen contraction (2.80 ± 0.45 sec.) and relaxation (71.40 ± 4.16 sec.) compared with clinically healthy buffaloes (Table 4).

In the affected buffaloes, the ruminal wall thickness (12.74 ± 1.87 mm), and the abdominal wall thickness (33.75 ± 3.3 mm) exhibited a significant increase than in clinically healthy buffaloes (7.85 ± 0.79 and 21.56 ± 1.43; respectively). On the 20th postoperative day, the affected buffaloes exhibited a non-significant change in the ruminal wall thickness (9.45 ± 0.79 mm) and abdominal wall thickness (26.87 ± 0.79 mm) compared with the clinically healthy buffaloes (Table 4).

4. DISUCSSION

To the best of our knowledge, there are no published reports on the occurrence or evaluation of rumino-cutaneous fistula in buffaloes.

Table 4 Ultrasonographic parameter differences between healthy control group and affected group with rumino cutaneous fistula before and after surgical interference

Items	Normal group	Before	Postoperative			
			3rd d.	10th d.	20th d.	30th d.
Rate of ruminal contraction (per 3 minutes)	3.80±0.71^{a}	1.20±0.45^{b}	1.40±0.55^{b}	1.60±0.55^{b}	2.40±0.55^{b}	3.00±0.55^{a}
Ruminal wall thickness (mm)	7.85±0.79^{c}	12.74±1.87 ^{a}	13.05±1.90 ^{a}	10.45±1.21^{b}	9.45±0.79^{bc}	8.85±0.49^{bc}
Abdominal wall thickness (mm)	21.56±1.43^{c}	33.75±3.30^{a}	34.63±3.21^{a}	31.00±1.53^{b}	26.87±0.79^{bc}	23.40±0.98^{c}
Duration of ruminal relaxation (second)	65.40±8.26^{d}	123.20±9.34^{a}	127.40±6.77^{a}	88.80±11.28^{b}	80.00±5.24^{bc}	71.40±4.16^{cd}
Duration of ruminal contraction (second)	2.40±0.55^{d}	5.00±0.71^{ab}	5.6±0.89^{a}	4.60±0.89^{b}	3.60±0.55^{c}	2.80±0.45^{cd}

The mean within the same row having different superscripts (letters) are significantly different at level ($p \leq 0.05$).

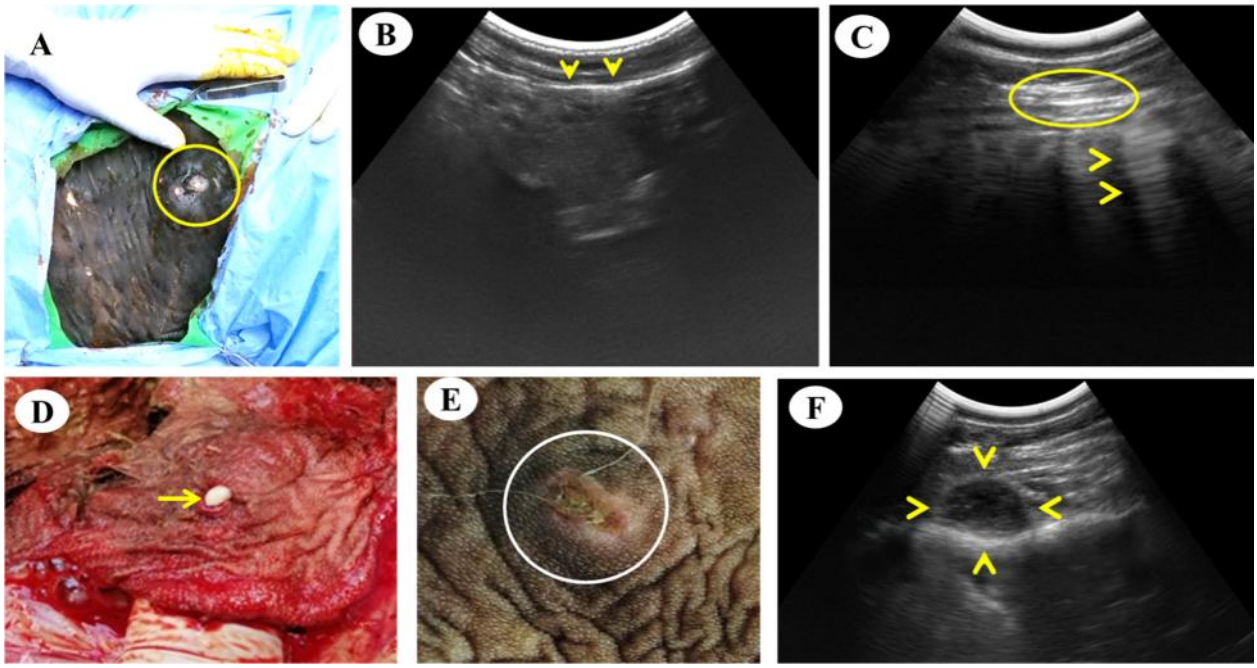

Figure 2. Evidence of several trocarization points in a buffaloe calf suffering from rumino-cutaneous fistula (yellow circle). (B) Ultrasonographic examination of the rumen in clinically healthy buffaloe revealed a thin echogenic wall lining (arrow heads) and underneath anechoic rumen content shadow due to gaseous contents. (C) Ultrasonographic examination of the fistulous tract in buffaloe suffering from rumino-cutaneous fistula revealed a hyperechoic bands (yellow circle) with acoustic shadowing (arrowheads). (D) Ruminal wall abscess (arrow) in buffaloe. (E) Stunted and sloughed ruminal papillae at the site of the fistula (white circle). (F) Ultrasonographic examination of ruminal wall abscess revealed an anechoic to hypoechoic indentation of the rumen wall (arrowheads) surrounded by an echogenic capsule.

Rumino-cutaneous fistula was recorded in an Ongole Cow (Prasad *et al.*, 2014) as a consequence of a penetrating wound on the left paralumbar fossa resulting from horn violence during fighting. Another 2 cases of ruminal fistula have been reported in 2 cow heifers (Singh *et al.*, 2014) due to unknown cause and the other one due to punctured wound by a fractured 9th rib. The results of the current study showed that the rumino-cutaneous fistula has been reported in buffaloes as a complication of rumenotomy and several attempts of rumen trocarization. Gastrointestinal disorders of reticulo-ruminal origin are characterized by general symptoms of indigestion, including anorexia, tympany, scanty stool, decreased milk production, and ruminal atony (Saini *et al.*, 2007; Abu-Seida and Al-Abbadi, 2014; Abdelaal and Floeck, 2015; Zeineldin et al., 2018). All affected buffaloes suffered from anorexia, recurring tympany, ruminal atony, scanty hard or mucoid soft stool, abdominal pain and regurgitation of food. These clinical symptoms were almost identical to those of acute local peritonitis in buffaloes as described by (Elgioushy *et al.*, 2019). In this study, gastrointestinal disturbances in animals with rumino-cutaneous fistulation could have been due to the accumulation of frothy tympany or indigestible food materials in the cranial sac of rumen, which could trigger the hypothalamus satiety center that controls feeding behavior (Ghurashi *et al.*, 2009; Aref and Abdel-Hakiem, 2013).

Buffaloes with ruminal fistula had a significant ($P \leq 0.05$) increase in rectal temperature, respiratory rate, and heart rate compared with clinically healthy control buffaloes. Similar results were reported in buffaloes with traumatic reticuloperitonitis (Abdelaal and Floeck, 2015). The significant increase in body temperature in affected buffaloes was linked to bacterial endotoxin and cytokine release due to bacterial infection (Aref and Abdel-Hakiem, 2013). In the affected buffalo, the substantial increase in respiratory and heart rates appeared due to the effect of elevated blood temperatures on the respiratory center and blood pressure (Omid and Mozaffari, 2014; Nongcula *et al.*, 2017).

In the present study, the affected buffaloes showed macrocytic hypochromic anemia that is characterized by significant increase in MCV with significant reduction in RBCs count, Hb content, HCT% and MCHC when compared with normal healthy ones. These results are in

concurrence with those obtained by Abdelaal et al., (2014). This observation may be due to inadequate dietary intake that resulted in deficiency in vitamin B12 or folic acid. This deficiency causes a slowing down of hematopoiesis due to reduced DNA synthesis that leads to production of large cells before division rich in hemoglobin. Moreover, it may be also due to the reduction in the absorption of vitamin B12 or folic acid as a result of the presence of foreign materials in the rumen (Vanitha *et al.,* 2010). This macrocytic hypochromic anemia was returned gradually to normal values of healthy buffaloes at the 30th postoperative day.

The affected buffaloes before the surgical operation revealed significant increase in total leukocyte count when compared with normal healthy group. This increase in total leukocyte count might be in response to stress, inflammation or localized and generalized infection (Athar *et al*., 2011; El-Ashker *et al.,* 2014). Meanwhile, this elevated level of total leukocytes was gradually decreased. The values were found to be normal at the 30th postoperative day which could be due to the removal of stress, infection and inflammation.

The liver enzymes ALT and AST were significantly elevated in the affected buffaloes when compared with normal healthy ones. This indicates the hepatocellular damage that is evidenced also by significant reduction in the serum total proteins and albumin when compared to normal healthy animals. These hypoproteinemia and hypoalbuminemia could be due to sequestration of plasma proteins into extravascular spaces such as body cavity effusions and vasculopathy or due to decreased production from liver, malnutrition, hepatic failure, malabsorption and maldigestion (Abdelaal *et al.,* 2009; Kusiluka, 2008; Aref and Abdel-Hakiem, 2013).After the operation, at the 30th postoperative day, the liver enzymes, serum total proteins and albumin retained to the normal level.

Renal insufficiency was recorded in the affected group when compared with normal healthy group which is manifested by significant increase in BUN and serum Creatinine with significant decrease in serum sodium and potassium levels. The decreased serum sodium and potassium levels may be also due to the inappetence or anorexia in these animals (Abdelaal *et al.,* 2009; El-Ashker *et al.,* 2014). Meanwhile, the buffaloes at the 30th postoperative day showed non-significant changes in BUN, serum sodium and potassium with significant decrease in serum Creatinine when compared with normal healthy animals.

Serum TNF-α is a key stimulator of hepatic production of acute phase proteins (Yoshioka *et al.,* 2002). In this study, we found a significant increase in TNF-α in animals before surgical operation when compared with both normal healthy animals and animals at the 30th postoperative day. The level of TNF-α was gradually decreased until it reached to the normal value at the 30th postoperative day. It was found that the increased level of serum TNF-α in our study was reflected by higher concentration in plasma acute phase proteins.

Acute phase proteins play an important role in physical and molecular responses that prevent inflammation and stimulate the processes of inflammation. They are synthesized in the liver during acute phase response. This acute phase response is initiated after trauma, inflammation, stress or infection (Cray *et al.,* 2009). Both fibrinogen and CRP are sensitive indicators of the severity of inflammation in bovine species (Jafarzadeh *et al.,* 2004). Fibrinogen is one of the positive acute phase proteins. The elevated plasma fibrinogen concentration in the buffaloes before the surgical operation when compared with the healthy ones was suggestive of tissue inflammatory process which is due to either traumatic, chemical or bacterial cause(Murata *et al.,* 2004; Athar *et al.,* 2011; Davolas and Akassoglo, 2012). The elevated level of the fibrinogen was returned to the normal values of healthy buffaloes at the 30th postoperative day. CRP has an important role in controlling the inflammatory response and to assess the individual animal stress. The increased level of CRP in the buffaloes before the surgical operation when compared with the healthy ones was indicative of tissue inflammatory process. The value of CRP is decreased gradually after the surgical operation, but its level remained more than normal value. This increased CRP values is due to the process of healing and resolution of the wound. The level of CRP evaluates the severity of inflammation (Cray *et al.,* 2009; Mold *et al.,* 2002).

A state of oxidative stress was recorded in all animals before the surgical operation which is manifested by significant elevation in the level of MDA when compared with normal healthy animals. The level of MDA was decreased gradually after the surgical operation until it reached to its normal level in the animals at the 30th postoperative day. MDA level is an indicator of lipid peroxidation that occurs after exposure to several reactive oxygen species (Kohen and Nyska 2002).

Ultrasonography is the method of choice for imaging and evaluation of rumen and ruminal wall physical and morphological characteristics (Blond and Buczinski, 2009). Ultrasonographic examination of rumen in the clinically healthy buffaloes was similar to the findings reported in other studies (Abdelaal *et al.,* 2009). The fistulous tract in the affected buffaloes displayed a hyperechoic band with acoustic shadowing, which is similar to findings recorded in a cow with reticular fistula (Blond and Buczinski, 2009; Abouelnasr *et al.,* 2012). Ultrasonographic examination of the surrounded rumen wall in affected buffaloes showed thicker echogenic particles of varying degrees of frequency punctuated by hypoechogenic fluid patches. Therefore, it was difficult to differentiate between the different layers of ruminal wall. Ruminal wall abscess was demonstrated in two buffaloes as anechoic to hypoechoic cavitation surrounded by an echogenic capsule with a diameter ranging from 0.4–0.8 cm. Similar findings have been reported in buffaloes with traumatic reticulitis and peri reticular abscess (Abouelnasr *et al.,* 2012).

Rumen contractions in the affected buffaloes were significantly decreased compared with clinically healthy buffaloes. This might be due to the adhesion between the ruminal wall and the surrounding organs (Vanitha *et al.,* 2010). In addition, reduced feed intake can lead to a reduction in chewing movement and passage of feed in the reticulum and rumen (Saini *et al.,* 2007).In the affected

buffaloes, the durations of rumen contractions and relaxation were significantly greater than in control buffaloes. Fibrous adhesions between the rumen and abdominal wall result in a delay in reticulo-ruminal contraction and extension of the duration of relaxation (Kumar *et al.,* 2014).

Compared with the clinically healthy buffaloes, the affected buffaloes exhibited a significant increase in the ruminal wall thickness and abdominal wall thickness. Similarly, buffaloes suffered from local peritonitis, and abdominal abscesses have been demonstrated to be associated with a substantial increase in the thickness of the reticulum as a result of the deposition of fibrinous tissue punctuated with fluid patches (Mostafa *et al.,* 2015).

5. CONCULOSIONS

In conclusion, rumino-cutaneous fistula is a common surgical complication of rumenotomy and rumen trocarization in buffaloes and a detailed diagnostic evaluation is necessary before and after surgical correction. Our study showed a significant improvement in postoperative clinical, hematobiochemical, and ultrasonographic parameters with minimal postoperative care without any further complications. Therefore, clinical and hematobiochemical examinations are of great value during the evolution of rumino-cutaneous fistula surgical correction; however, additional diagnostic tools such as ultrasonography is important for proper assessment of ruminal morphological and physical characteristics.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest to disclose.

ACKNOWLEDGMENT

Sincere thanks are particular to Prof. Dr. Hussein M. El-Maghrapy for his valuable support and continuous help during article reviewing. We also sincerely thank the crew of veterinary hospitals and educational ruminant farm at college of Veterinary Medicine, Benha University for help during the sample collection and surgical operation.

STUDY LIMITATION

Small sample size and different age, weight and sex of animals were the points limiting this study.

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