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CLINICAL AND HAEMATOBIOCHEMICAL EVALUATION OF DIARRHEIC NEONATAL BUFFALO CALVES (BUBALAS BUBALIS) WITH REFERENCE TO ANTIOXIDANT CHANGES

Ghanem, M.M.[a,*], El-Fkhrany, S.F.[b], Abd El-Raof, Y.M.[a], El-Attar, H.M.[a]
[a] *Animal Medicine Department, Faculty of Veterinary Medicine, Benha University,* [b] *Public Health and Common Diseases, Directorate of Veterinary Medicine, Menoufia*

ABSTRACT

The present study was carried to investigate the prevalence, etiology and changes related to enteritis and diarrhea in newborn buffalo calves (n=100) of both sexes from birth up to 3 months of age. The prevalence of diarrhea was 60% and the mortality rate was 10%. E. coli (73.3%) and salmonella (26.7%) were isolated from diseased calves. Based on clinical examination and skin fold test; calves were divided into three groups. Group 1 (control): apparently healthy calves (n=10, selected from 40). Group 2: calves suffering from mild diarrhea (n=10, selected from 38). Group 3: calves suffering from severe diarrhea (n=10, selected from 22). SOD significantly reduced in both mild and severe diarrhea compared to control. A strong negative correlation was found between the degree of diarrhea and the levels of Cu, Zn and Fe, respectively. Besides, there were a significant elevation in liver enzymes (ALT, AST& ALP) and kidney function test (BUN& creatinine). Histopathology of intestine of diarrheic calves revealed thickening of mucosa and wall, and occasionally desquamation or atrophy of lining epithelium. The mesenteric lymph node showed expansion of the medullary sinuses by increased numbers of inflammatory cells mainly large macrophages, lymphocytes along with fibrin. It was concluded that high prevalence (60%) of diarrhea in newborn buffalo calf associated with haematological, biochemical and histopathological changes. Additionally, results signified the relationship between Cu, Zn and Fe deficiency and the degree of diarrhea. The reduction of SOD highlights the role of oxidant injury in induction of enteritis in newly born buffalo calves.

KEY WORDS: Buffalo calves, Biochemical, E. coli, Enteritis, SOD

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1. INTRODUCTION

Diarrhea is a major problem in livestock production in Egypt and throughout the world [22]. Gastro enteritis in newborn calves causes high morbidity and mortality, leading to significant economic losses in Egypt [6]. Neonatal diarrhea is a major source of economic loss in the cattle industry and a leading cause of calf mortality in most countries. Calf scours is not a single disease entity; it is a clinical syndrome associated with several diseases characterized by diarrhea. Regardless of the cause, absorption of fluids from the intestine is altered, leading to life-threatening electrolyte imbalances. The scouring calf loses fluids rapidly dehydrated and suffers from electrolyte loss and acidosis. Infectious agents may cause initial damage to the intestine, but death from scours usually results from dehydration, acidosis, and loss of electrolytes. Identification of infectious agents that cause scours is essential for implementation of effective preventive and treatment measures [33]. Enteritis and

* Corresponding author: e-mail: dr.mohamedghanem@yahoo.com, mohamed.ghanem@fvtm.bu.edu.eg

diarrhea are leading cause of calves' death during their first weeks of life. Wide range of causative infectious agents may be involved in this pathology. In general, the main causes are bacterial infections, viral and protozoan agents can infect the animals [11]. The E. coli diarrhea (Scour) in newborn calves (9-10 days of age) is usually characterized by watery white or yellowish diarrhea, rapid onset and time course, and high mortality. In affected calves, diarrhea typically begins with 36 to 72 hours after birth and calves die within 2 to 3 days [26, 33]. Rotaviruses were major cause of diarrhea in reared farm animals throughout the world [28]. The most common protozoa agents responsible for the diarrhea are Cryptosporidium, Eimaria and Giardia [19]. The bovine coccidiosis could be produced by several Eimeria species, of which E. bovis and E.zuernii are the most pathogenic [18]. The clinical symptoms observed in diarrheic calves were manifested by loose stools, a lack of appetite and abdominalgia, persistent diarrhea results in dehydration, severe weakness and gradual loss of suckling reflex [38]. The pH value of blood in neonatal diarrheic calf below 7 and HCO3 concentration of 20.0mmol/L are reflective of metabolic acidosis. The diarrhea can lead to dehydration, acidemia, primarily due to strong ion (metabolic) acidosis, hyperkalaemia, and impaired cardiovascular and renal functions. In addition, metabolic acidosis in diarrheic calves is originally attributed to fecal bicarbonate loss as well as the presence of unidentified organic acids in plasma and a decrease in glomerular filtration rate in response to severe dehydration and renal ischemia [10, 23]. The clinical symptoms are accompanied by a variety of metabolic disorders manifested by changes in enzymatic and biochemical indices [37]. Hematological parameters as well as the antioxidant activities are affected in previous studies [7, 17, 20].

Therefore, the aim of this study is to: determine the prevalence of neonatal diarrhea in buffalo calves, record the clinical picture associated with diarrhea in buffalo calves, determine the hematological and biochemical changes that accompany the gastro-enteritis in buffalo calves and use hematological, serum biochemical findings and field tests as prognostic indicators for the severity of calf diarrhea and dehydration, establish the relationship between diarrhea in calves and the antioxidant injury, establish the relationship between trace element deficiency (especially copper, zinc, and iron) and diarrhea in calves, and isolate and identify the causative agents causing buffalo calves diarrhea.

2. MATERIALS AND METHODS

2.1. *Animals*

The present study was carried on 100 newly born buffalo calves of both sexes from 2 days to 3 months of age that were fed on whole milk in a private farm in menoufia governorate. Based on the initial clinical examination, 40 calves were apparently healthy and 60 were suffered from different degrees of diarrhea. We selected 30 calves for further examination. According to the skin fold test, capillary refill time and clinical examination, calves were classified into three groups:

Group 1 (control group; n=10): included apparently healthy calves fed on whole milk.

Group 2 (mild diarrhea; n=10): included calves fed on whole milk and suffered from mild diarrhea.

Group 3 (severe diarrhea; n=10): included calves fed on whole milk and suffered from severe diarrhea.

2.2. *Clinical examination of animals*

Segregation of diseased calves and examination were carried out carefully using the methods described by Radostits et al. [56].

2.3. *Field diagnosis of dehydration in buffalo calves*.

2.3.1. *Skin fold (tent) test*
Skin fold (tent) duration was measured in seconds at the lateral portion of the thorax over the 6^{th} to 9^{th} for one sec. The skin fold test is performed by tenting or twisting a fold of skin at the neck or thorax over the 6^{th} to 9^{th} ribs by 90 degree for 1 sec and then allowed to release. The skin tenting was monitored for 10 sec.

2.3.2. *Capillary refill time (CRT)*
The capillary refill is the rate at which blood refills empty capillaries. It can be measured by pressing on the gum of the animal by the finger until it turns white, and taking note of the time needed for the color to return once pressure is released. Normal refill time is less than 2 seconds. The capillary refill time (CRT) is a common indication of dehydration and decreased peripheral perfusion. The process whereby blood returns to apportion of the capillary system after its blood supply has been interrupted briefly. Capillary refilling is tested by pressing firmly on gum and estimating the time required for blood to return after pressure is released.

2.3.3. *Palpebral reflex*
palpebral reflex test is done by touching the medial or lateral canthus of the eye and observing for a blink .This reflex is mediated by the trigeminal nerve (CN V, Facial sensation) and the facial nerve (CN VII, closure of the eye).

2.3.4. *The degree of enophthalmos (sunken eye)*
Enophthalmos was defined as a visible gap between the eye ball and caruncula lacrimalis. The extent of enophthalmos was quantified by measuring the distance (in mm) between the medial canthus and the eye ball.

2.4. *Isolation and identification of causative agents*
One fecal sample was taken in a clean dry plastic packs for parasitological examination to detect gastro intestinal parasites [17] and the second using sterile swabs for further bacteriological analysis. These swabs were immediately inoculated on Carry and Blair's transport medium and were cultured on selective and differential culture media at 37ºC for 24 hours and the isolated colonies were then identified. Isolated colonies from MacConky's agar plate were examined to be either lactose fermenting or non-lactose fermenting. Lactose fermenting colonies appeared to be rose pink in color and non-lactose fermenting as pale yellow colonies. Isolated colonies were then examined by Gram staining (Gram negative bacilli).

2.5. *Hematological examination*
The total erythrocytic count (TEC), total leukocytic count (TLC), mean corpuscular volume (MCV), hemoglobin (Hb), packed cell volume (PCV%), lymphocytes %, monocytes %, granulocytes % were measured by using hematology analyzer [35].

2.6. *Biochemical analysis*
Spectrophotometric assay using special commercial kits and Spectrophotometer (SpinLab, Spinreact S.A. Model 2003) were used for determination of glucose [68], urea [54], creatinine [71], calcium [65], phosphorus [72], magnesium [29], sodium and potassium [33], chloride [5], copper and iron [5], zinc [25], AST [15], ALT [69], ALP [58], total protein [53], albumin [26]. Globulin was determined by the differences between total protein and albumin [16]. A/G ratio was calculated by dividing the albumin over globulin [26].

2.7. *Serum protein electrophoresis*
The serum protein electrophoresis was conducted by the separation of charged molecules according to their movement under the influence of applied electric field [36, 43].

2.8. *Serum antioxidant activities*

Serum Super oxide dismutase (SOD) as indicator for antioxidant activity was determined by using special kits as previously described [51].

2.9. *Histopathological examination*
Samples taken from intestines were collected immediately after sacrifice, fixed in neutral buffered formalin 10%, dehydrated, and embedded in paraffin. The paraffin blocks were sectioned at 5-7µ thickness, and stained with Haematoxylin and eosin (H&E) as previously recorded [64].

2.10. *Statistical analysis*
The data represented as means (±SE) were statically analyzed by one way analysis of variance (ANOVA) as previously described [52] using Microsoft excel 2010. Differences were considered significantly different from control healthy when $P \leq 0.05$. Correlation coefficient (r) between Cu, Zn and Fe for and the degree of diarrhea was tested.

3. RESULTS

3.1. *Prevalence of neonatal calf affected with diarrhea*:
The prevalence of neonatal calf diarrhea were 60% where 60 calves out of 100 calves examined showed different degrees of diarrhea of the 60 calves affected, 38 calves (38 %) had mild degree of diarrhea and, 22 calves (22 %) demonstrated severe type of diarrhea while mortality rate was 10% (Table 1).

Table 1 Prevalence of neonatal diarrhea in buffalo calves.

Total examined	Apparent. Healthy		Diseased calves				Mortality	
	No	%	Mild diarrhea		Severe diarrhea		n	%
			n	%	n	%		
100	40	40	38	38	22	22	10	10

3.2. *The clinical signs*:
The common clinical signs appeared on the control group were normal appetite, clear shiny eyes, bloomy coats, and the mean body temperature, pulse and respiratory rates were 38.5 °C, 95 beats per minute and 25 breaths per minute, respectively. In the 2nd and 3rd groups calves had thin and watery feces, signs of dehydration (sunken eyes, dry mucus membranes, and rough hair) (Fig. 1), calf extremities were cold to the touch, loss of appetite, difficulty in getting up, tendency to lie down and unable to rise with loss of consciousness (Table 2).

3.3. *Field diagnosis of dehydration in buffalo calves*.
In healthy calves the average duration of skin fold test was 3 sec while in calves with mild and severe diarrhea the average duration was significantly prolonged (6 and 10 sec, respectively). The capillary refill time was increased in calves with mild and severe diarrhea (3 and 5 sec, respectively) compared to healthy calves (1 sec). In palpebral reflex, eyelids are closed immediately and fully when touching by finger in healthy buffalo calves, whereas in mild diarrheic buffalo calves, eyelids are closed with delay and not fully.

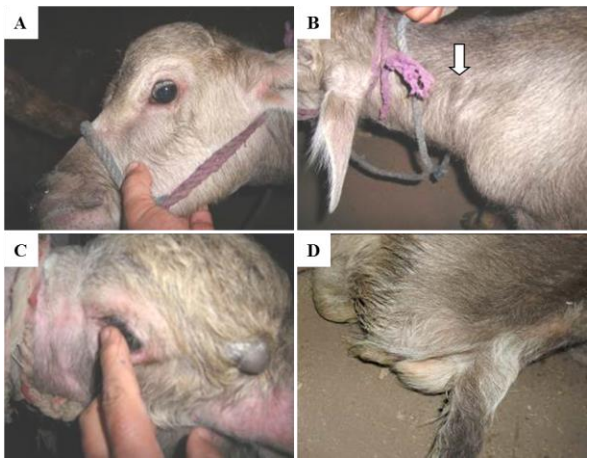

Fig. 1 21-days old buffalo calf with severe diarrhea showed signs of dehydration; sunken eye, separation of eyeball and orbit (A), delayed skin fold test (B) and eyelids are not closed at all (palpebral reflex test) (C). Fig. 1D 5-days old buffalo calf showed soiling of perineum and tail with yellow feces.

In severe diarrheic buffalo calves, eyelids are not closed at all. Eye examination showed clear skinning eyes in healthy buffalo calves with no gap between eye ball and orbit whereas mild affected calves had slightly sunken eye (small gap between eye ball and orbit) and severely affected calves had Severe sunken eyes (large gap between eye ball and orbit) (Table 3).

3.4. *Bacteriological results*:
Bacteriological examination of the fecal samples of diarrheic buffalo calves revealed that 60 samples were positive for pathogenic bacteria (Table 4). The percentage of infection was 73.3% E. coli and 26.7% salmonella.

3.5. *Hematological examination*:
Hematological changes showed significant increase in PCV, WBCs, MCV and non-significant increase in neutrophil %, lymphocytes %, monocytes% and MCHC. On other hand, there were significant decrease in RBCs, MCH and pH (Table 5).

3.6. *The biochemical analysis*:
The biochemical analysis of mild and severe diarrheic buffalo calves showed significant ($p \leq 0.05$) decrease in Ca, P, Mg, Cl and Na and showed significant ($p \leq 0.05$) increase in K (Table 6). There was a significant ($p \leq 0.05$) decrease in Zn, Cu and Fe in mild and severe diarrheic buffalo calves (Table 7). There were significant elevation in liver enzymes (ALT, AST), ALP and kidney function test (urea and creatinine) (Table 8). There was significant ($p \leq 0.05$) decrease in glucose in buffalo calves with mild and severe diarrhea compared to control (Table 9).

Table 2 Clinical examination of healthy and diarrheic buffalo calves.

Groups	------ General signs ------		---------- Alimentary signs ----------			Degree of enophthalmos	Palpebral reflex
	Appetite	Demeanor (behavior)	Diarrhea	Intestinal motility	Skin exam.		
Clinically healthy	Normal	Adequate reaction to stimuli, very bright and alert	Semi solid feces of light green color	Normal	Bloomy and elastic skin	Clear skinning eyes	Eye lids are closed immediately and fully
Mild diarrhea (pasty feces)	Mild anorexia	Mild (Calves suppression response only to painful stimuli)	White-yellow fetid, profuse watery	hypermotility	Mild dehydration	Slightly sunken eye (small gap between eye ball and orbit)	Eye lids are closed with deadly and not fully
Severe diarrhea (fluidy feces with discoloration)	Severe anorexia	Sever suppression (No reaction to stimuli)	White-yellow offensive and watery bloody tinged	hypermotility	Moderate to severe dehydration	Severely sunken eyes (large gap between eye ball and orbit).	Eye lids are not closed at all

Table 3 Changes in temperature, pulse and respiratory rates, skin fold test and capillary refill time in buffalo calves with mild and severe diarrhea compared to control group.

Parameter	Control group (n=10)	Diarrheic groups	
		Mild (n=10)	Severe (n=10)
Temperature (°C)	38.46±0.05	39.52±0.22	37.20 ±0.50*
Pulse rate (Beats/min)	95.00 ± 0.40	80.6±2.48	60.8±13.76*
Respiratory rate (Breaths/min)	24.80 ± 0.64	31.2±1.04	38 ±1.20*
Skin fold test (Sec)	3±1	6±2*	10±3*
Capillary refill time test (Sec)	1±1	3±2	5±3*

*means significant change from control at $P \leq 0.05$.

Table 4 Bacteriological examination of fecal samples of diarrheic buffalo calves.

Total examined (diseased calves)	Causative organism	n	% of infection
60	E. coli	44	73.3
	Salmonella species	16	26.7

Table 5 Changes in hematological value in buffalo calves with mild and severe diarrhea compared to control group.

Parameter	Control group (n=10)	Diarrheic groups	
		Mild (n=10)	Severe (n=10)
PCV (%)	25.86 ±2.97	28.56 ±0.23*	37.88 ±4.30 *
Hb (g/dl)	8.54 ±0.11	10.74±0.95*	11.36 ±1.51*
RBCs (10^6/µI)	11.16 ±0.11	10.37 ±0.21*	8.29 ±1.01 *
WBCs (10^3/µl)	9.08 ±1.63	11.61 ±0.62*	15.09 ±1.9 *
MCV (Fl)	23.17 ±3.43	27.54 ±1.3 *	45.69 ±3.77*
MCH (Pg)	13.76 ±1.05	8.94 ±1.74 *	8.82 ±1.06 *
MCHC (%)	33.02 ±9.07	37.60 ±3.6	29.98 ±2.55*
Lymphocytes (%)	44.75 ±6.63	53 ±5.2	57.8 ±4.56
Monocytes (%)	1.25 ±0.3	1.8 ±0.32	1.9 ±0.32
Neutrophil (%)	45.8 ±8.24	48 ±4.4	52 ±3.8 *
pH	7.38±0.05	7.05±0.08	6.7±0.1*

*means significant change from control at $P \leq 0.05$

Table 6 Changes in serum minerals and electrolyte in buffalo-calves with mild and severe diarrhea compared to control group.

Parameters	Units	Control group	Diarrheic groups	
			Mild	Severe
Ca	mg/dL	11.42 ±0.29	9.11 ±0.19*	8.33 ±010*
P	mg/dL	7.11 ±0.23	4.68 ±0.11*	3.57 ±0.31*
Mg	mg/dL	1.83 ±0.07	1.44 ±0.02*	1.33 ±0.03*
K	mmol/L	4.3 ±0.24	5.96 ±0.49*	7.05 ±0.22*
Cl	mmol/L	93.58 ±0.95	83.53 ±0.75*	74.18±0.85*
Na	mmol/L	134 ±4.00	119.2 ±3.04*	119.8 ±1.76*

*means significant change from control at $P \leq 0.05$

Table 7 Correlation between the levels of Cu, Zn and Fe the degree of diarrhea and enteritis.

Parameter	Diarrheic group			Correlation Coefficient (r)
	Control (n=10)	Mild (n=10)	Sever (n=10)	
Cu (mg / dl)	139.4±3.92	126.16±12.41	119.8±1.76*	- 0.98
Zn (mg / dl)	197.16±13.71	148.38±12.41*	132.86±0.17*	- 0.95
Fe (mg / dl)	203±7.60	148.2±2.24*	131.8±7.04*	- 0.93

Table 8 Changes in serum liver enzymes and antioxidant activities (SOD) in buffalo-calves with mild and severe diarrhea compared to control group.

Parameters	Units	Control group	Diarrheic groups	
			Mild	Severe
ALT	IU/L	64.2 ±2.64	78.4 ±4.32*	99.2 ±4.96*
AST	IU/L	85.00±2.8	122.6±12.64*	150.6±3.92*
ALP	IU/L	56.32±0.91	130.2±9.4*	254.4±13.28*
SOD	U/Ml	424±26.4	353.2±3.04*	310.8±1.76*

* means significant change from control at $P \leq 0.05$

On the other hand, there were non-significant ($p \leq 0.05$) increases in total protein, significant increase in albumin, significant decrease in globulin and significant increase in A/G ratio in mild and severe cases (Table 10). There was non-significant decrease in α-1 globulin and α-2 globulin but β and γ globulins were significantly ($p \leq 0.05$) decreased in mild and severe than control group (Table 11).

3.7. *The histopathological examination*:
The histopathological changes of intestine of mild and severe diarrheic calves showed reduction of the columnar epithelium to cuboidal and occasionally squamous epithelium. The crypt epithelium was damaged and failure of replacement of absorptive cell and the villous epithelium is attacked and there is accelerated loss of absorptive cells, also there is shrinkage (atrophy) of villi. The small intestine mucosa of the neonatal calf consists of long finger-like villi and when challenged by a variety of infectious agents the pathological lesion consists of also stunting and thickening of the villi, frequently the villi are fused leading (Fig. 2&3).

Table 9 Changes in serum kidney function tests and glucose level in buffalo-calves with mild and severe diarrhea compared to control

Parameters	Units	Control group n=40	Diarrheic groups	
			Mild n=30	Severe n=30
BUN	mg/dl	26.57±4.92	31.22±5.38*	36.68±6.08*
Creatinine	mg/dl	175.96±17.7	181.7±4.04*	189.44±11.7*
Glucose	mg/dl	97.74±6.8	63.74±8.77*	51.34±1.51*

* means significant change from control at $P \leq 0.05$

Table 10 Changes in serum proteins value in buffalo-calves with mild and severe diarrhea compared to control group.

Parameters	Units	Control group (n=40)	Diarrheic groups	
			Mild (n=30)	Severe (n=30)
Total protein	gm/dL	7.57±0.10	7.86±0.08	8.07±0.08
Albumin	gm/dL	4.06±0.24	4.39±0.24*	4.57±0.22*
Globulin	gm/dL	3.51±0.054	3.21±0.36*	2.80±0.29*
A/G Ratio	ratio	1.15±0.08	1.36±0.21*	1.63±1.19*

*means significant change from control at $P \leq 0.05$

Table 11 Changes in serum protein electrophoresis value in buffalo-calves with mild and severe diarrhea compared to control group.

Parameters	Units	Control group	Diarrheic groups	
			Mild	Severe
Alpha 1 globulin	g/dL	0.83±0.03	0.77±0.04	0.42±0.027
Alpha 2 globulin	g/dL	0.89±0.026	0.82±0.05	0.75±0.04
Beta globulin	g/dL	0.84±0.028	0.65±0.054*	0.60±0.051*
Gamma globulin	g/dL	1.20±0.098	0.94±0.058*	0.90±0.059*

*means significant change from control at $P \leq 0.05$

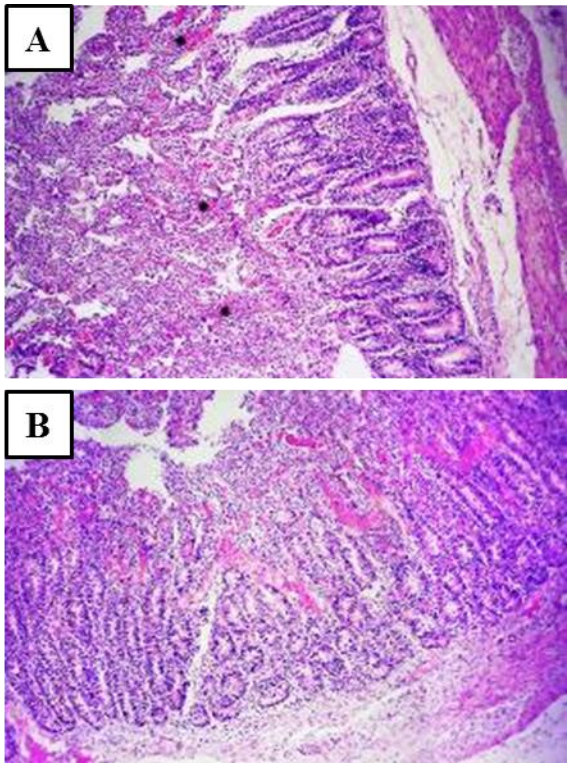

Fig. 3 Intestine of a calf had severe enteritis characterized by thickening of the mucosa and wall (A) and well-demarcated areas of coagulative necrosis within the superficial mucosa (B) stained with H&E stain (×100).

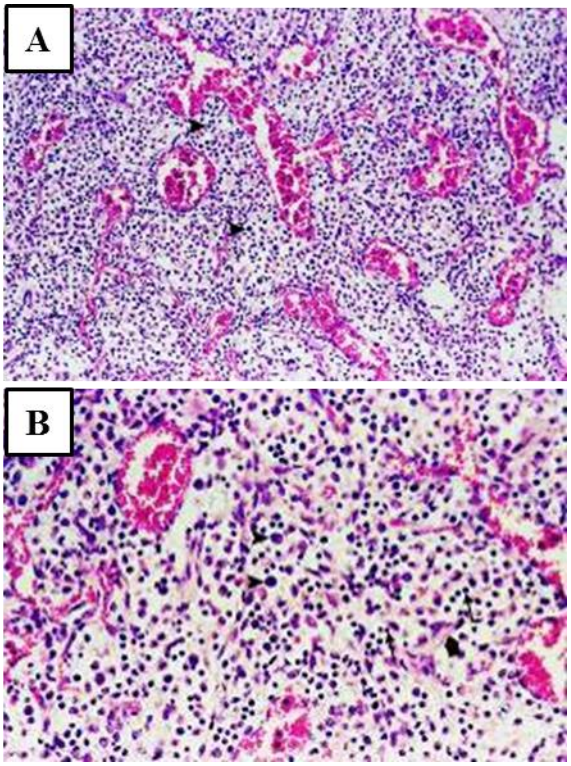

Fig. 4 Mesenteric lymph node of a calf showed medullary sinuses expanded by increased numbers of inflammatory cells (A; ×200) mainly large macrophages (arrow head), lymphocytes (arrow), along with fibrin (thick arrow), and edema (B;×400) stained with . H&E stain.

5. DISCUSSION

The prevalence of neonatal calf diarrhea was 60%; of which 38% suffered from mild diarrhea and 22% suffered from severe diarrhea. This result is similar that previously observed [15]. Recent studies found that the prevalence of newborn calf diarrhea is 53.6% [45]. The mortality rate was 10% that was nearly similar to that previously recorded [24]. The common clinical signs in calves affected with diarrhea include depression, dullness and deprived appetites. As well as soiling of hind quarter with feces. Nearly similar findings were observed by Abd-Elrahman [1]. The clinical signs appeared on the mild diarrheic calves were the ability to stand without assistant and had well to moderate suckling affinity while in severe diarrhea, they were always observed in sternal recumbency, lethargic with weak or no suckling affinity to lateral recumbency with complete or incomplete loss of consciousness [29-31, 34]. The severity of dehydration could be assessed clinically according to the degree of the skin elasticity and the degree of the sunken eyeball. Mild diarrheic neonate calves usually exhibited mild skin tenting skin return to normal in 4 seconds and no significant changes in eyeball, while severely diarrheic neonatal calves showed with severe dehydration as indicated by severe decrease in skin elasticity skin fold return to normal in 7 seconds and moderate to severe sunken eyeball. Nearly Similar results were recorded by previous study [14, 60]. Moreover, the severity of dehydration can be assessed by capillary refill time [27]. In our study mild diarrhea calves had capillary refill time in 6±3 sec. while in severe diarrhea the capillary refill time is prolonged for 10 second. These results coincided with those previously reported [27]. Infectious diarrhea is a common condition affecting newly born buffalo calves in this study. Fecal samples screened the presence of the common enteropathogenic organisms (E. coli and

Salmonella species) which causing diarrhea. E. coli seems to be the dominant enteropathogen which plays the major role among diarrheic buffalo calves. We found that the percentage of E. coli infection was 73.6 % and Salmonella was 24.4 %. These results are nearly similar to findings of previous studies [1, 11, 41, 46, 56].

Haematological examination demonstrated significant increase in PCV in mild and severe diarrhea that was similar to other studies [2, 36]. Increased PCV% indicates excessive loss of body fluid and inadequate intake of milk and fluids during diarrhea [5, 7, 20]. The significant decreased in RBCs count in mild and severe than control could be attributed to the long standing diarrhea and dehydration and hem concentration [5, 35]. There was significant increase in MCV in mild and severe diarrhea, and this may be attributed to production of immature RBCs of large size [4]. There was significant decrease in MCH of mild and server than control, and this may be attributed to loss of blood and low level of hemoglobin in erythrocytes. The significant increase in WBCs count in mild and severe diarrhea than control might be attributed to the infection by microorganisms and attributed mainly to neutrophilia [20, 27].

Metabolic acidosis was detected in newborn buffalo calves with diarrhea as there was a significant decrease in blood pH in mild and server diarrhea compared to control, a result that matches many other studies [12, 66]. This result may be attributed to fecal bicarbonate loss, as well as the presence of unidentified organic acids in plasma, and a decrease in glomerular filtration rate in response to severe dehydration [10].

Serum analysis of mild and severe diarrheic buffalo calves showed significant ($P<0.05$) decrease in Ca, P, Mg, Cl, Na, Zn, Cu, Fe, SOD, and significant ($P<0.05$) increase in K in mild and severe than control. The decrease in Ca level might be attributed to persistent diarrhea and dehydration with loss of Ca in feces [16, 36]. The decrease in P was attributed to greater electrolyte loss than water loss [21]. The low serum Mg might be attributed to decreased absorption or diarrhea Also the low serum Cl and Na were attributed to loss of large amounts of Cl and Na ions related to increased intestinal secretion and diarrhea [14]. The significant ($P<0.05$) decrease in Cu, Zn, and Fe levels might be attributed to decrease in absorption of food nutrient through the intestine and losses in feces [2, 39].

Serum liver function tests of mild and severe diarrheic buffalo calves showed significant ($P<0.05$) increase in ALT and AST. This result might be attributed to chronic inflammation of gastrointestinal tract of diarrheic calves and pathological affection of the liver and digestive tract [13]. The significant increase in ALP level might be due to damage of intestinal mucosa, progressive inflammatory process, and release of the intestinal fraction of the enzyme to blood circulation [40].

Serum analysis of mild and severe diarrheic buffalo calves showed significant ($P<0.05$) decreased in the mean values of super oxide dismutase enzyme (SOD) level than the control. This result may be attributed to stress condition related to diarrhea [1, 2, 16, 25]. Since SOD degrades the superoxide into oxygen and hydrogen peroxide which are less toxic substances, its low level leads to accumulation of oxidant substances and free radical that caused cellular damage to the intestinal lining mucosa. Therefore, the decreased SOD in enteric calves highlights the role of oxidative stress in the pathogenesis of enteritis in neonatal calves. It could also a result of hypocuppremic occurring in diarrhea where Cu is the major activator of SOD [25]. This result signifies the importance role of antioxidants as a therapeutic agent during prescription drugs for enteritis in neonatal calves.

Serum kidney function tests of mild and severe diarrheic buffalo calves showed significant ($P<0.05$) increased in the mean

values of blood urea nitrogen (BUN) and creatinine level compared to the control. The increase in serum blood urea nitrogen (BUN) levels might be attributed to deficit in renal blood perfusion (glomerular filtration rate) and reduced urine formation [35]. It could also attributed to excessive production of urea by catabolism of body proteins in severe toxic conditions [9]. All dead diarrheic neonate calves exhibited a high level in BUN and serum creatinine [5].

Serum analysis of mild and severe diarrheic buffalo calves showed significant ($P<0.05$) decreased in glucose level than the control. The hypoglycaemia might be attributed to lack of glucose absorption from damaged intestine [3]. Protein profile analysis showed non-significant increase in total protein, significant increase ($P<0.05$) in albumin and significant decrease in beta and gamma globulin in mild and severe diarrhea than control diarrheic buffalo calves [20]. The decreased in mean values of gamma (γ) globulin in mild and severe diarrheic buffalo calves may be attributed to failure of calves to receive adequate quantity of colostrums after birth or due to action of the pathogenic agents on the immune system leading to suppressing of the response to any pathogenic agent [2]. Since gamma globulin represents the humeral immunity of calves the results indicate that enteritis in calves is stress-related disease

Histopathologestic examination of intestinal tract of mild and severe diarrheic buffalo calves showed the damage of the crypt epithelium and failure of replacement of absorptive cell and the villous epithelium was attacked. Also there was a shrinkage (atrophy) of villi and reduced absorptive capacity leading to an accumulation of fluid in the intestine due to reduced absorption of active secretion and changes in pressure gradient which leads to scours [8, 12, 32].

In a conclusion, the severity of diarrhea in neonatal calves could be classified into mild and severe degree based on the field tests, such as skin fold test, capillary refill time, sunken eye degree and palpebral reflex that proved reliable diagnostic values. The prevalence of neonatal gastroenteritis in neonatal buffalo calves is high (about 60%) which suggest that special care must be given to calves during this critical age till 3 months of age. Enteritis caused acid base and electrolyte imbalance, haematological changes and histopathological changes. Gastroenteritis in neonatal calves is associated with reduction of Zn, Fe and Cu level and related to oxidant injury diagnosed by reduction of the antioxidant SOD. The reduction in gamma globulin in calves with diarrhea suggests that special care must be given to neonatal calves to make sure that they receive their colostrum soon after parturition and given plasma globulin as compensatory drugs to avoid diarrhea.

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مجلة بنها للعلوم الطبية البيطرية
BENHA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

التغيرات السريرية، الدموية، والبيوكيميائية في عجول الجاموس الرضيعة المصابة بالاسهال مع التركيز على الأنشطة المضادة للأكسدة

محمد محمدى غانم[1]، صبري فريد نبوى الفخراني[2]، ياسين محمود عبد الرؤوف[1]، حسام الدين محمد عبد العزيز العطار[1]

[1] قسم طب الحيوان (الأمراض الباطنة)- كلية الطب البيطرى- جامعة بنها.

[2] الصحة العامة والامراض المشتركة -مديرية الطب البيطري بالمنوفية.

الملخص العربي

أجريت هذه الدراسة على عدد مائة من عجول الجاموس الرضيعة حديثة الولادة من كلا الجنسين خلال الفترة من الولادة حتى ثلاثة أشهر. كان معدل انتشار الإسهال 60٪ و معدل الوفيات هو 10%. بالفحص البكتريولوجى وجد ان مسببات الاسهال الأكثر شيوعا في عجول الجاموس كانت الاشيريشيا كولاي (73,7%) والسالمونيلا (26,3%). تم اختيار 30 عجل لمزيد من الدراسة قسمت استنادا إلى الفحص الاكلينيكى واختبار ثنى العجول الى ثلاث مجموعات متساوية العدد. المجموعة الأولى (المجموعة الضابطة): شملت العجول الاصحاء ظاهريا ولا يعانون من الاسهال. المجموعة الثانية: مجموعة الاسهال الخفيف. المجموعة الثالثة: مجموعة الاسهال الشديد. وتم تقييم مدى خطورة الإسهال في من خلال تقييم درجة الجفاف والحموضة، مرونة الجلد، مدى الغور في مقلة العين، درجة حرارة الجسم ومعدل التنفس، ومعدل ضربات القلب. إشتملت التغيرات الدموية على زيادة في PCV٪، وعدد الكريات البيضاء والهيموجلوبين وMCH و MCHC ودرجة حموضة الدم ونقص فى كرات الدم الحمراء. أما التغيرات البيوكيميائية اشتملت على نقص الكالسيوم، الفوسفور ,الصوديوم، الكلوريد، الماغنسيوم وانخفاض معنوى فى مستوى النحاس، الحديد، الزنك، ومستويات السكر في الدم و مستوي السوبر اوكسيد ديسميوتيز، و انخفاض الانزيمات الكبدية أيضا (ALT،AST، ALP)، و وظائف الكلى (BUN، الكرياتينين). كما لوحظ وجود علاقة عكسية بين درجة خطورة الاسهال ومستويات النحاس والحديد والزنك. وكشف الفحص الهستوباثولوجى للامعاء وجود ضمور الخلايا الطلائية المبطنة لأمعاء العجول ذات الاسهال المعتدل والشديد. اثبتت هذه الدراسة ان الإسهال يحدث بنسبة عالية فى عجول الجاموس الرضيعة (60%) ولكن بدرجات متفاوتة وهناك علاقة بين مضادات الاكسدة ومستوى العناصر النادرة وحدوث الاسهال فى العجول.

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