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Clinical and Haemato-biochemical Changes of Parasitic Enteritis in Buffalo with Reference to Acute Phase Protein

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Abstract

This study was conducted on adult and calf buffaloes for recording the clinico-hematobiochemical alterations of parasitic enteritis. Diarrheic buffaloes showed depression, dullness, depraved appetite, variable degrees of diarrhea, cessation of rumination, labored breathing, accelerated heart and respiratory rates, weakness and a reluctance to move. As diarrhea progressed, dehydration of the affected buffalo was evidence with signs of alopecia. Hematological analysis showed significant decrease($p<0.05$) in RBCs, Hb, PCV and WBCs. Serum biochemical analysis revealed significant decrease ($p<0.05$) in sodium, chloride, copper, zinc and iron glucose and SOD levels in diarrheic buffaloes. While, there was significant increase in serum potassium and haptoglobin levels in diarrheic buffaloes compared with control. Therefore, we concluded the parasitic enteritis was associated with alteration in hematology and serum micronutrient as well as the oxidative stress indicated by lowered SOD.

Keywords: Buffalo**,** Haptoglobin, Parasitic diarrhea, SOD, Trace element .

1. Introduction

Enteritis and diarrhea constitute the most serious problem among farm animals which lead to considerable economic loss**.** It is one of the major problems facing animal production especially those reared under intensive or semi-intensive system of breeding; because of the significant mortalities of young (s), cost of treatment, weight loss or even retarded growth[14] . The etiology of diarrhea is multiple, including infectious agents, poor management, reproductive factors, nutritional factors and immune depression[10] . The diarrheic animals lose fluid, rapidly dehydrated and suffered from electrolyte loss, acidosis. The infectious agents may cause initial damage to the intestine but death from scours usually results from dehydration, acidosis and loss of electrolytes [26] . Worms and coccidiosis commonly cause diarrheas in recently weaned calves. In gastro-intestinal nematodes group of parasites have a remarkable status as the main pathogens causing severe damage to their hosts because their blood-sucking feeding habits causing anemia that can be so sever resulting in death of the animals[20] . Oxidative stress has been implicated as a major initiator of tissue damages and can affect enzymatic activity, signal transcription and gene expression, especially apoptotic gene in animals suffering from stressful conditions, due to parasitism[3] . The acute phase response (APPs) is the reaction of the animal to disturbances in its homeostasis caused by infection, tissue injury, neoplastic growth, or immunological disorders APPs may provide an alternative means of monitoring animal health. An increased focus on the application of APP for this purpose has recently been developed[17] . Some studies have been conducted regarding APPs concentrations in calves with diarrhea [24] . Therefore, this study was conducted to record the clinical and hematobiochemical changes in diarrheic buffalo with special reference to antioxidant status and acute phase protein.

2. Materials and methods

2.1 Experimental animals

The present study was carried out on 120 adults and calf's buffaloes with different ages and sexes from different localities belonged to different breeding farms in Qalubia Governorate in addition to the animals admitted to the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Benha University. The animals suffered from diarrhea due to various causes. This study was conducted in the period from the beginning of 2014 till the end of 2015. The animals were examined clinically to describe the clinical evidence of diarrhea and the presence of parasites. Based upon age, buffaloes were classified into two groups, group of adult and group of calves. In calves group, 10 animals were suffered from parasitic enteritis and 10 were control healthy. In the second group, 10 adult buffaloes were suffered from parasitic enteritis and 10 were control.

2.2 Samples

2.2.1 Fecal samples

Fecal samples were taken directly from the rectum for parasitological examination to detect different forms of gastro intestinal parasites[9] . The fecal examination was conducted as previously described [31].

2.2.2 Blood samples

The blood samples were collected from jugular vein of all animals in the examined groups by using cleaned and dry sharp needle. The blood was allowed to flow freely and gently from the vein in a clean dry and labeled container. Two blood samples were collected from each animal, one with EDTA for hematological examination and one without anticoagulant for biochemical analysis.

2.3 Clinical examination of animals

Examination of diseased animals was carried out using the methods described previously [26] **.**

2.4 Hematological examination

The red blood cell count (RBCs), total leukocytic count (TLC), hemoglobin (Hb) and packed cell volume (PCV %) were determined by using automatic cell counter.

2.5 Biochemical analysis of serum

Serum glucose was determined spectrophotometrically by using of special kits (Biodiagnostic, Egypt) according to the method that described previously [33].

2.5.1 Serum macro and micro elements

Serum potassium and sodium, serum chloride, serum iron and copper and serum zinc were determined spectrophotometrically by using of the special kits (Biodiagnostic, Egypt) according to [35,36,37,19,34,5,13] respectively.

2.5.2 Serum Alkaline phosphatase (ALP)

ALP was determined spectrophotometrically by using of the special kits .[28]

2.5.3 Serum Super oxide diamutase (SOD)

SOD was determined by using of the special kits (Biodiagnostic, Egypt) [22].

2.5.4 Serum acute phase protein (haptoglobulin (Hp)

APP was measured using validated standard methods. Hp was measured based on prevention of the peroxidase activity of hemoglobin, which is directly proportional to the amount of Hp [32] .

2.6 Statistical analysis

The data were statistically analyzed using T-test (student test) to compare diseased group to the corresponding control group of the same age as previously described[23] . We used Microsoft excel 2010 to conduct this analysis. Values were represented as means ± standard error (SE). Differences were considered significantly different from control healthy when $P< 0.05$.

3. Results

3.1 Clinical signs

The most prominent clinical signs among the buffalo with enteritis (diarrheic) were mild to severe diarrhea, depression, dullness and depraved appetite. Diseased animals showed variable degree of diarrhea, these animals also were suffering from one or more of the following signs: elevated in body temperature (40-41°C), loss of appetite, cessation of rumination, labored breathing, accelerated heart and respiratory rates, weakness and a reluctance to move are the symptoms developed especially in more protracted cases, as shown in Fig (1) . Calves showed the signs of severe diarrhea which was profuse watery to pasty feces, usually pale yellow to greenish in color and occasionally streaked with blood and foul smelling. Defecation was frequent and effortless soiling the tail and buttocks. As diarrhea progressed, dehydration of the affected calves occurred and calves become very weak, completely anorexic and lying down with alopecia Fig (2), severely sunken eyeball and pale conjunctival mucous membranes. However, there was a slight variation in clinical signs showed by the animals in the present study Table (1).

3.2 Results of coprological examination of fecal samples

Coprological examination of fecal samples revealed that the total prevalence of enteritis in buffaloes was 60% (72/120). The total parasitic diarrhea infection rate was 48.6% (35/72), where 26.38% in calves (19/72) and 22.22% in adult (16/72). The total non-parasitic enteritis had 51.4% (37/72), where 18.05% in calves (13/72) and 33.33% in adult (24/72), (as shown in Table (2).

3.3 Incidence of different types of internal parasites in diarrheic animals

Fecal examination results indicated that the infection among calves was about 54.29% (19/35) and 45.71% (16/35) in adult Table (3).

3.4 Hematological examination

The obtained hematological examination of apparent healthy control groups and diarrheic buffaloes groups are described in Table (4), where the levels of packed cell volume (PCV), total red blood corpuscles (RBCs), hemoglobin (Hb), total white blood cells (WBCs) showed significant

decrease in diarrheic buffaloes compared with corresponding values in healthy groups.

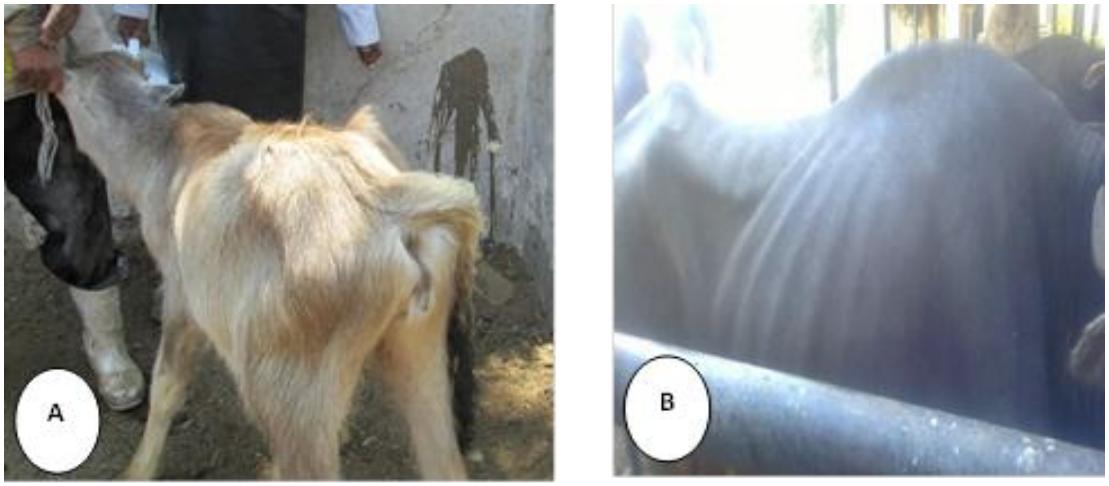

Fig (1) Adiarrheic buffalo calf (A) and Adiarrheic adult buffalo (B) showing signs of emaciation and reluctance to move.

Fig (2) A diarrheic buffalo calf showing signs of severe emaciation, rough coat (A) and signs of alopecia (B).

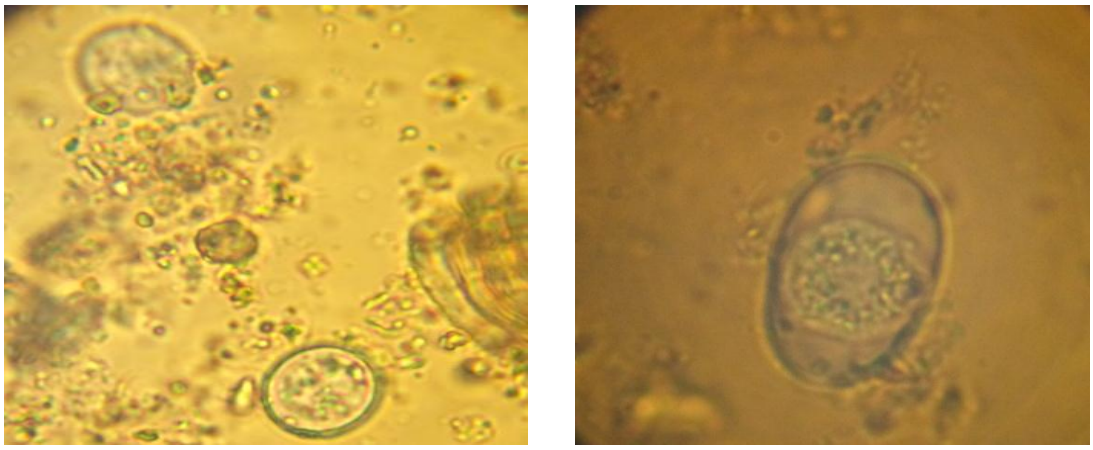

Fig (3) Microscopical examination of fecal samples showing different species of non sporulated Eimeria eggs (A and B).

Table (1) Percentage of different clinical signs in buffaloes affected with enteritis

Clinical sings	Number of affected animals/total	% sick ones
Diarrhea	72/72	100 %
Fever	18/72	25%
Anorexia	63/72	87.5%
Cessation of rumination	21/72	29.2%
Dullness / depression	35/72	48.6%
Pale mucous membrane	13/72	18.1%
Emaciation	45/72	62.5%
Sunken eyes	12/72	16.7%
Alopecia	5/72	6.94%

Table (2) Prevalence of parasitic and non- parasitic diarrhea in examined buffaloes

Age	Parasitic diarrhea		Non-parasitic diarrhea	Total diseased
Adult buffalo	No.	16	24	40
	% .	22.22%	33.33%	55.6%
Buffalo Calves	No.	19	13	32
	%	26.38	18.05%	44.4%
Total number		35	37	72

No. = number of examined animals. % = Percent.

Table (3) Incidence of internal parasites recovered in diarrheic animals

Animal	Buffalo calves		Adult buffaloes		Total diseased
Type of Parasite	**No**	**%**	**No**	**%**	
Egg of nematode	3	8.57%	4	11.43%	7
Nematode, Eimeria,	5	14.28%	3	8.57%	8
Nematode, Fasciola	2	5.71%	0	0	2
Nematode, Eimeria, Fasciola	1	2.86%	1	2.86%	2
Eimeria oocysts	5	14.28%	3	8.57%	8
Fasciola egg	1	2.86%	2	5.71%	3
Cryptosporidia	0	0	1	2.86%	1
Monesia	1	2.86%	1	2.86%	2
Trichuris	1	2.86%	0	0	1
Segments of cestodes	0	0	1	2.86%	1
Total number	19	54.29%	16	45.71%	35

Table (4) Changes in hematological value in buffalo with diarrhea compared to control group (Mean ± S.E)

Groups	Units	Group I		Group II	
Parameter		Control calves (n=10)	Diseased calves (n=10)	Control adult (n=10)	Diseased adult (n=10)
PCV	%	36.0 ± 0.58^{b}	26.25 ± 0.48^{a}	36.75 ± 0.48^{b}	27.67 ± 0.53^{a}
Hb	gm/dl	10.85 ± 0.202^{a}	5.40 ± 0.953^{b}	9.37 ± 1.594^{a}	6.74 ± 1.03^{b}
RBCs	$10^{6}/\mu l$	7.50 ± 1.33^{a}	$1.47 \pm .0198^{b}$	5.14 ± 1.699^{a}	2.55 ± 0.714^{b}
WBCs	$10^{3}/\mu l$	8.30 ± 2.18^{a}	$4.41 \pm .741^{b}$	8.35 ± 0.087^{a}	$3.35 \pm .065^{b}$

Mean values with different superscripts are significantly different from corresponding control values at $P < 0.05$.

3.5 Biochemical analysis of serum

The biochemical analysis of serum macro and micro elements of apparent healthy (control) and diarrheic buffaloes (Table5) showed significant decrease in sodium, chloride, copper, zinc and iron levels in diarrheic buffaloes groups compared with corresponding values in healthy control groups while there is significant increase in potassium level in diarrheic calves than in healthy ones. Where the serum alkaline phosphatase (ALP) showed significant increase while the level of superoxide dismutase (SOD) showed significant decrease in diarrheic buffaloes groups compared with corresponding values in healthy control groups

Table (6). On the other hand, the serum glucose levels showed significant decrease while haptoglobin showed significant increase in diarrheic buffaloes groups compared with corresponding values in healthy control groups.

Table (5) Changes in serum minerals and electrolyte in buffalo with diarrhea compared to control group (Mean ± S.E)

Groups	Units	Group I		Group II	
Parameters		Control adult (n=10)	Diseased adult (n=10)	Control calves (n=10)	Diseased calves (n=10)
Chloride	mmol/l	90.0 ± 1.15[a]	69.3 ± 0.88[b]	88.0 ± 0.58[a]	66.67 ± 5.2[b]
Sodium	mmol/l	120.5 ± 2.5[a]	115.5 ± 5.5[b]	129.5 ± 2.02[a]	123.5 ± 2.5[ab]
Copper	mg/dl	40.5 ± 0.87[a]	26.67 ± 2.4[b]	40.5 ±0.29[a]	22.67± 1.4[b]
Zinc	mg/dl	48.0 ± 2.3[a]	32.3 ± 5.21[a]	48.0 ± 0.58[a]	37.67 ± 2.4[ab]
Iron	mg/dl	125 ± 3.46[a]	85 ± 2.08[b]	122 ± 0.58[a]	82.33 ± 2.4[b]

Mean values with different superscripts are significantly different from corresponding control values at $P < 0.05$.

Table (6) Changes in serum ALP, SOD, glucose and haptoglobin value in buffalo with diarrhea compared to control group (Mean ± S.E

Groups	Units	Group I		Group II	
Parameters		Control adult (n=10)	Diseased adult (n=10)	Control calves (n=10)	Diseased calves (n=10)
ALP	iu/l	78.0 ± 3.02[b]	210 ±5.07[a]	81.5 ± 0.29[b]	186 ±3.62[a]
SOD	u/ml	14.0 ± 0.58[a]	6.17 ± 0.46[b]	14.7 ± 0.11[a]	6.67 ± 0.67[b]
Glucose	gm/dl	91.75± 12.64[a]	68.36 ± 9.99[b]	99.0 ± 1.73[a]	62.33 ± 5.93[b]
Haptoglobin	mg/dl	123.5 ± 3.17[b]	286.33 ± 2.90[a]	123.5 ± 0.87[b]	265.67 ± 2.73[a]

Mean values with different superscripts are significantly different from corresponding control values at $P < 0.05$.

4. Discussion

Our result showed that prevalence of diarrhea in the examined buffaloes were 60% where 72 animals out of the total 120 animals examined showed different degrees of diarrhea where 32 buffalo calves (26.7%) and 40 adult buffaloes (33.3%) demonstrated different degree of diarrhea. This result was similar to that reported[11] . Diarrhea constitutes one of the most serious problems among farm animals which lead to considerable economic loss. The diarrheic animals showed variable degree of diarrhea, depression, dullness and depraved appetite. Nearly similar finding were observed by[1] . The faeces was semi fluid to watery in consistency and grayish to yellowish green in color, contained mucous and sometimes blood. The perineum and tail were soiled with feces. These results come in agreement with those previously recorded by[12] . The recorded anorexia, depression and dullness may be attributed to the fever while the muscular weakness due to escape of intracellular potassium, hyperkalemia and hypoglycemia [26].Severely diarrheic animals showed severe dehydration as indicated by severe decrease in skin elasticity and moderate to severe sunken eyeball. Moreover, alopecia may be attributed to mineral deficiencies, as zinc deficiency. Severe Zn deficiency may contribute to skin changes, including peeling, scabbing, itching and hair loss. These changes are symptoms of parakeratosis, a disorder which is probably caused by impaired protein synthesis and activation of Zn-dependent enzymes that are essential for carbohydrate, lipid and nucleic acid metabolism [4]. Coprological examination of fecal samples revealed that the total prevalence of GI parasitic infections in diarrheic buffaloes was 48.6% (35/72) of which, 26.38% in calves (19/72) and 22.22% in adult (16/72) (Table 2). The observed increase in prevalence of GI parasitic infections in diarrheic buffaloes may be attributed to sexual reproduction of GI nematodes that leads to release of eggs through urine or feces into the environment. These eggs or resulting larval transmission stages reinfect the host by oral ingestion or by skin penetration. The most striking features of many GI nematodes are their long-term persist (1-10 years) within the host despite immune recognition, their ability to evade host defenses, and their ability to continually reinfect the host, all of which contribute to their classification as long-lasting chronic infections [21] . Moreover, these results were nearly similar to that recorded by [38]) who found that in dairy calves with liquid

feces showed 34.8+ 4% incidence rate of combined helminths and Eimeria spp. The decrease in PCV was similar to that observed by [29] who reported that there was decrease in PVC in calves with enteric and septicemic E. coli infection. In addition, [6] and [3] reported that there was decrease in PCV % in buffalo calves suffering from coccidiosis. The hemoglobin (Hb) content showed significant decreased in diarrheic buffaloes compared with corresponding values in control ones. This result agreed with [3] and [16] who mentioned that there was a decreased in Hb that attributed to hemoconcentration and the loss of body fluid and electrolytes in extreme diarrheic and dehydrated condition in buffalo calves infected with T. vitulorum and may be attributed to anorexia and diarrhea. The total red blood corpuscles (RBCs), showed significant decrease in diarrheic buffaloes compared with corresponding values in control ones. These results agree with [6,3] who reported a decrease in RBCs count in buffalo calves suffering from coccidiosis with dehydration and diarrhea This result may be attributed to diarrhea and anorexia. In addition, [6] also explained the reduction in RBCs which might be attributed to the blood loss from the hemorrhagic intestinal mucosa and bloody diarrhea. The total white blood cells (WBCs) count, showed significant decrease in diarrheic buffaloes compared with corresponding values in healthy groups. The decrease of total leucocyte count in diarrheic animal may be attributed to the stress of malnutrition. This suggestion was supported by the result obtained by [14].Also this result agrees with [29] who reported that there was decreased in WBCs in dehydrated calves with diarrhea. But it disagrees with those observed [16] who reported that there was increased in WBCs than the control.

Serum analysis of diarrheic buffalo showed significant decrease in the sodium (Na) level than the control. This result agreed with [30,16,12]. The decrease in serum Na levels in diarrheic buffalo may be attributed to the loss of large amounts of Na ion with intestinal secretion and diarrhea. [30] reported that a significant decrease in the concentration of Na and Cl ions in the blood of diarrheic calves due to their loss with feces. The decrease in serum chloride levels in diarrheic buffalo calves may be attributed to the loss of large amounts of chloride ion with intestinal secretion and diarrhea [16] .

Serum analysis of diarrheic buffalo showed significant decrease in the iron (Fe) level than the control. This result agreed with[15] . This result may be attributed to loss of iron in feces in Eimeriosis infected calves[3] . Serum analysis of Cu in diarrheic buffalo showed significant decrease in serum Cu level than the control groups. This result may be attributed to the effect of gastrointestinal parasites on copper metabolism by interference with copper absorption from the gastrointestinal tract by increasing the pH of the gastric environment [2].This result agreed with[3,12,15] .

Serum analysis of zinc (Zn) level in diarrheic buffalo groups showed significant decrease than the control. This may be attributed to damage of intestinal epithelium caused by enteritis. This result agreed with [15,27] who stated blood zinc concentration in diarrheic claves was significantly lower than the healthy animals due to poor absorption and loss of zinc in the gastrointestinal tract during diarrhea to increased demand for zinc by the immune system and to utilization and sequestration of zinc at the tissue level for synthesis of antioxidant enzymes as a compensatory mechanism to counter excessive free radical production.

Serum analysis of diarrheic buffalo animals showed significant increase in the potassium (K) level than the control. This result was coincided with agreed with [30,16,15] who observed an increase in the level of potassium ion in neonatal diarrheic calves which has a direct relationship with disturbances in the acid-base balance. An increased pH value in serum results in a decreased concentration of potassium ions. In contrast, its decrease leads to an increased concentration of these ions. The mechanism of these interactions consists in the exchange of hydrogen and potassium ions between the extracellular and cellular spaces. This exchange may be evoked by the primary increase in the concentration of hydrogen ions (pH drop) in the cellular liquid, and results in their shift to cells. In accordance with the rule of electric inertness of systemic fluids, the potassium ions shift in an opposite direction (from a cell to extracellular fluid), thus causing an increase in their plasma concentration.

Serum alkaline phosphatase (ALP) (Table 6) showed significant increase in diarrheic buffaloes groups compared with corresponding values in healthy groups. This result agreed with [41,18] where the level serum alkaline phosphatase (ALP) was increased, may be due to damage of intestinal mucosa, progressive inflammatory process, and release of the intestinal fraction of the enzyme to blood circulation []

The superoxide dismutase (SOD) showed significant decrease in diarrheic buffaloes groups compared with corresponding values in healthy groups. This implies that the affected calves are under stress condition due to parasitism. This was coincided with [3] . The decreased SOD in enteric calves high lights the role of oxidative stress in the pathogenesis of enteritis in calves. It could also a result of hypocuppremic occurring in diarrhea where Cu is the major activator of SOD [39] 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.

The serum glucose level showed significant decrease in diarrheic buffaloe group than control groups. This result agreed with [16,15]. The decreased in serum glucose levels (hypoglycemia) in buffalo calves suffering from diarrhea may be attributed to lack of glucose absorption from damaged intestine. Moreover, the alterations in tissue metabolism caused by decreased blood flow and oxygenation associated with shock [40]

The serum haptoglobin level showed significant increase in diarrheic buffaloes than control groups. This result agrees with [25,8,17,7] , as Hp concentrations correlated better with disease severity (fever, anorexia, general weakness, loss of condition, moderate and severe profuse watery diarrhea and dehydration).

In conclusion, parasitic infestation can be considered one of the major causes of diarrhea in buffalo. Hence, parasitic diarrhea is associated with alteration in nutritional elements in the diarrheic buffaloes. It is recommended to add the micronutrients to the prescriptions for treatment of the parasitic enteritis. In addition, addition of antioxidant is important during of treatment of the parasitic enteritis.

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