Benha Veterinary Medical Journal 39 (2020) 90-94

Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

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

Since 1990

Original Paper

Clinical and biochemical investigations on bacterial diarrhea in Egyptian buffalo calves

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ARTICLE INFO

Keywords

Biochemical
Buffalo calves
Clinical
Diarrhea

Received 04/12/2020
Accepted 22/12/2020
Available On-Line 20/01/2021

ABSTRACT

The objective of the present study was to evaluate clinico-biochemical changes occurred in bacterial diarrhea in Egyptian Buffalo calves less than three months old. The study was carried out on 50 buffalo calves in El-Galaa Military farm that located in Ismailia Governorate, 40 diarrheic calves aged 55-75 days with an average body weight (35±2.5) kg were detected and 10 apparently healthy calves were kept as a control group to investigate the clinico-biochemical profiles. E. coli mixed or co-infection of Acinetobacterbaumannii/ Iwoffiiss species with Klebsiella and pseudomonas species were isolated from the diarrheic calves. Diarrheic calves show signs of anorexia, weakness, dullness, staggering movement, weight loss, pale mucous membranes with sunken eyes and dehydration. Analysis of clinical and biochemical profile of the diarrheic calves revealed significant increase in body temperature, respiratory rate, skin fold test, serum potassium, chloride, urea, creatinine, ALT, AST, GGT, triacylglycerols, cortisol, CRP and CPK with significant decrease in pulse rate, serum sodium, glucose and total protein concentrations. It could be concluded that, bacterial diarrhea has a severe negative impact on the clinical and biochemical profile of the affected buffalo calves

1. INTRODUCTION

Calf diarrhea is the main reported disease in Buffalo Calves and has major concerns due to the economic losses to cattle industry in which those losses not only from mortality but also from other costs as treatment, diagnostics, labor, veterinary intervention and decreased number of herd replacements as well as subsequent chronic ill thrift and impaired growth performance (Bazeley, 2003). Calf diarrhea is a multifactorial syndrome which occurs due to both non-infectious and infectious factors (Izzo et al., 2011). Non-infectious factors as insufficient uptake of colostrum, high/low milk feeding, stress, poor sanitation or cold weather which all lower the defense mechanism within calves during early life and become vulnerable to infectious factors (Muktar, 2015). Infectious factors causing diarrhea include viruses, bacteria, and protozoa. Among those agents; bovine corona virus (BCoV), bovine rotavirus (BRV) group A and bovine viral diarrhea virus (BVDV) as viral agents. Salmonella species, Escherichia coli (E.coli) and Clostridium species as bacterial agents and Cryptosporidium spp. as a protozoan agent. In addition, co-infection of multiple pathogens is very common in calves with diarrhea (Bhat et al., 2013).

Although the cattle industry has made great improvements with herd management, animal facilities and care, feeding and nutrition, and timely use of bio-pharmaceutics, calf diarrhea is still an obstacle due to the multi-factorial nature of the disease. Prevention and control of calf diarrhea should be based on a good understanding of the disease complexities such as multiple pathogens, co-infection, environmental factors, and feeding and management during the calving period before disease outbreaks (Cho and Yoon, 2014).

Serum biochemical analysis of diarrheic calves revealed a significant increases in Albumin: Globulin (A:G) ratio, urea, albumin, creatinine and potassium concentrations. However, the values of serum glucose, sodium and chloride concentrations were markedly decreased (Singh et al., 2014). Clinical application of acute phase protein in large ruminant has not been sufficiently standardized in routine practice. However, acute phase protein response develops novel application during some less frequent studies diseases such as young diarrhea (Tóthová et al., 2013). The research results indicated that there was an increase in the level of C-reactive protein in piglets suffering from catarrhal enteritis (Rychlik et al., 2001).

Accordingly, the clinical picture and serum biochemical analysis in diarrheic Egyptian buffalo calves were evaluated. Besides, faecal bacteriological examinations to identify the bacterial causative agent(s) were also investigated.

2. MATERIAL AND METHODS

2.1. Animals:

A total number of 50 buffalo calves in El-Galaa Military farm that located in Ismailia Governorate were examined, 40 diarrheic calves aged 55-75 days old with an average live body weight (35±2.5) kg were detected and 10apparently healthy calves with no history of any previous illness were kept as a control group. The animals under the study has been on a vaccination program that includes bovine viral

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diarrhea vaccine, rift valley fever vaccine, three-day sickness vaccine, foot and mouth disease vaccine, one shot ultra-vaccine, pneumovax vaccine. Calves at the first day of age has been vaccinated with calf guard® which act against rotavirus and coronavirus infections.1.5±0.5 kg of milk was supplied for the buffalo calves twice daily by milk bottle feeding system. Ad Libitum balanced concentrates and Alfalfa hay were used to supply the buffalo calves with normal requirements of proteins, fats, minerals and vitamins (Table 1).

Table 1 Analysis of the balanced diet used in the farm:

Analysis of ingredients	%
TDN	69.65
CP	14.3
CF	11
Mg	0.3
Ca	0.95
P	0.40
Na	0.42
CL	0.58
Energy	2800
Fat	2.6

2.2. Clinical examination of animals

Body temperature, respiration, pulse rate and skin fold test were carried out using the methods described by Constable et al. (2016)

2.3. Sampling

2.3.1. Blood samples

Blood samples for serum separation were collected from jugular vein of each calf in a sterile tube then centrifuged at 3000 rpm for 10 min, then clear non-hemolyzed sera were obtained and transferred into clean, dry and sterilized Eppendorf tubes then kept at -20^{0}c till used for biochemical analysis.

2.3.2. Fecal samples

Fecal samples were obtained from each animal for analysis by digital rectal stimulation with a gloved hand in a sterile specimen container and were immediately inoculated on Carry and Blair's transport medium.

2.3 2. Parasitological examination:

Fecal samples were taken in a dry sterile specimen container for parasitological examination to detect gastrointestinal parasites according to method described by Zajac et al. (2012); in which results were negative of any fecal parasite.

2.4. Bacteriological examination:

Faecal samples from healthy and diarrheic buffalo calves were inoculated and cultured on selective and differential culture media at 37^{0}C for 24 hours and the isolated colonies were then identified and counted of pathogenic bacterial causative agent of diarrhea (ISO 4832:2006-E) (ISO 4833-1:2013).

2.5. *Biochemical analysis*

Serum calcium (Ca), Inorganic phosphorus (P), Chloride (Cl) , Potassium (K) , Sodium (Na), urea and Creatinine were determined spectrophotometrically according to the methods described by Tietz, (1986), Cockayne and Anderson (1993), Henry et al. (1974) , Jansen et al. (1991), Patton and Crouch (1977) and Hout (1985), Patton and Crouch (1977) and Hout (1985), respectively.

Moreover, Liver marker enzymes [Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Gamma-Glutamyl Transferase (GGT)], Total protein, albumin, glucose, total cholesterol and triacylglycerols were determined according to Thefeld (1974), Ritman and Frankel (1957) and Breuer (1996), Pagana (2017), Young (1990), Allain et al. (1974), respectively. In addition, serum cortisol and C-reactive protein (CRP) concentrations were determined using ELISA kit (Eucardio Laboratory, Inc., Encinitas, and CA., U.S.A.) and creatine phosphokinase (CPK) was determined by the method of (Rec. GSCC 1977).

2.6. Statistical analysis:

The obtained results from the experiments were expressed as mean ± SEM and were analyzed by T-test using (SPSS Statistics for Windows, version 25.0. Armonk, NY: IBM Corp). Differences were declared significant when ($P < 0.05$).

3. RESULTS

3.1. Clinical findings:

Anorexia, Weakness, dullness, staggering movement, weight loss, pale mucous membranes with sunken eyes and watery diarrhea with foul odor (colored greenish, milky white, blackish or yellowish) were the prominent clinical signs in most diarrheic calves. Moreover, signs of dehydration of diarrheic calves were recorded. There was a significant ($p< 0.05$) increase in body temperature, respiration and skin fold test (Table 2); with a significant ($p< 0.05$) decrease in pulse rate. In advanced cases calves showed complete recumbency, incoordination and in ability to stand or suckling. During the study two of diarrheic calves were died showed signs of profuse watery yellowish diarrhea, severe dehydration (prolonged skin fold tent), complete lateral recumbency, labored breath, hypothermia and bradycardia prior to death.

3.2. Biochemical results

Serum Na and Cl levels were significantly ($p< 0.05$) decreased while serum K level was significantly ($p< 0.05$) increased in the diarrheic calves compared to healthy calves (Table 3).

In diarrheic calves, serum urea, creatinine, ALT, AST and GGT levels were significantly ($p< 0.05$) increased while serum albumin, glucose and total protein concentrations showed significant ($p< 0.05$) decreases compared to healthy calves. While serum triacylglycerols exhibited significant ($p< 0.05$) increase (Table 4). In addition, serum cortisol, CPK and CRP levels were significantly ($p<0.05$) increased in the diarrheic calves compared to healthy calves (Table 5).

3.3. Bacteriological findings

Pathogenic E. coli isolated from the fecal samples from 22 diarrheic calves which represented 55% of the diarrheic calves (Table 6) with a bacterial count (30.16 x 10^5 CFU\gm) (Table 7).

Table 2 Body temperature, respiratory rate, pulse rate and skin fold test in healthy and diarrheic Egyptian buffalo calves.

Animal groups	Body temperature (°C)	Respiratory rate (cycle\min)	Pulse rate (pulse wave\min)	Skin fold test (sec)
Control Group (n=10)	38.2 ± 0.05 ^{b}	24.86 ± 0.34 ^{b}	94 ± 0.31 ^{a}	3.6 ± 0.29 ^{b}
Diarrheic Group (n=40)	40.1 ± 0.17 ^{a}	32.72 ± 0.88 ^{a}	77 ± 1.77 ^{b}	6.3 ± 0.30 ^{a}

Data are presented as (Mean ± SE). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

Table3 Alterations of serum electrolytes, minerals, and urea and creatinine concentrations in healthy and diarrheic Egyptian buffalo calves.

Animal groups	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)	Ca (mg/dl)	I.P (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Control group (n=10)	150.4 ± 2.42 [a]	4.87 ± 0.006 [b]	107.44 ± 2.5 [a]	9.98 ± 0.32	5.40 ± 0.23	27.24 ± 1.4 [b]	0.64 ± 0.02 [b]
Diarrheic group (n=40)	132.32 ± 1.57 [b]	7.01 ± 0.04 [a]	78.85 ± 2.15 [b]	9.94 ± 0.19	5.28 ± 0.15	46.86 ± 2.19 [a]	1.21 ± 0.16 [a]

Data are presented as (Mean ± SE). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

Table 4 Alterations of some serum biomarkers in healthy and diarrheic Egyptian buffalo calves.

Animal groups	ALT (U/L)	AST (U/L)	Albumin (g/dl)	GGT (U/L)	Glucose (mg/dl)	Total Protein (g/dl)	Total Cholesterol (mg/dl)	Triacylglycerol's (mg/dl)
Control Group (n=10)	36.98 ± 1.8 [b]	98.05 ± 2.7 [b]	3.61 ± 0.06 [a]	13.98 ± 1.29 [b]	105.95± 3.9 [a]	7.72 ± 0.14 [a]	85.98 ± 2.04	56.51 ± 1.35 [b]
Diarrheic Group (n=40)	77.67 ± 3.14 [a]	155.61 ± 2.1 [a]	3.15 ± 0.08 [b]	41.26 ± 3.97 [a]	90.74 ± 3.1 [b]	6.82 ± 0.08 [b]	85.46 ± 1.78	62.04 ± 1.56 [a]

Data are presented as (Mean ± SE). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

Table 5 Alterations of Serum Cortisol, CPK and CRP in healthy and diarrheic Egyptian buffalo calves.

Animal groups	Cortisol (U/L)	CPK (U/L)	CRP (mg/dl)
Control Group (n=10)	46.16 ± 2.6 [b]	225.28 ± 4.9 [b]	12.61 ± 0.78 [b]
Diarrheic Group n=(40)	104.12 ± 3.8 [a]	367.29 ± 9.6 [a]	62.08 ± 4.39 [a]

Data are presented as (Mean ± SE). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

Table 6 Bacteriological examination of fecal samples from diarrheic Egyptian buffalo calves.

Bacterial isolate	Total Number of +ve samples	Isolation percentage in diarrheic group
Pathogenic E. Coli	22	55%
Acinetobacterbaumannii/Iwoffiiss Spp. mixed with klebsiella	14	35%
Pseudomonas spp	4	10%

Furthermore, mixed or co-infection of Acinetobacterbaumannii/Iwoffiiss species with Klebsiella has been isolated from 14 diarrheic calve which represented 35% of faecal samples from diarrheic calves; while the bacteria was significantly absent in the healthy calves (Table 6). As well Pseudomonas spp. was isolated from 4 diarrheic calves which represented 10% of fecal samples from diarrheic calves; while the bacteria were significantly absent in the healthy calves (Table 6).

Table 7 Pathogenic E. Coli bacterial count of fecal samples from healthy and diarrheic Egyptian buffalo calves.

Animal groups	(CFU\gm)
Control Group (n=10)	<100
Diarrheic Group (n=40)	30.16×10^5

4. DISUCSSION

The prevalence of calf diarrhea is one of the most common diseases in young animals causing huge economic and productivity losses to bovine industry worldwide (Cho and Yoon 2014).The recorded age in the study was 55-75 days old as diarrhea is the most common disease reported in calves up to three months old (Svensson et al., 2003).

There is multiple etiology of diarrhea and the importance of microbiological diagnosis relating to the research of different enteropathogens of diarrhea in calves to better understand their etiology, and help in the development of a specific treatment (Langoni et al., 2004). Bacteriological examination in the current study showed 55% of the diarrheic calves had pathogenic E. coli isolated from the fecal samples that is previously reported by El-Seedy et al. (2016) as infectious diarrhea is a common condition affecting young calves as Salmonella species and E. coli K99+ are known as the most common pathogens identified in scouring calves. Other fecal samples estimated about 35% of the diarrheic calves had a mixed or co-infection of Acinetobacterbaumannii / Iwoffiiss Spp. with Klebsiella. Additionally, 10% of the diarrheic calves had Pseudomonas spp. As it is very common in calf diarrhea cases where more than one of the pathogenic agents is present which was similar to previous studies (Muktar et al., 2015).

In the present study, clinical signs appeared as mainly the presence of diarrhea, which was watery, mucoid, contains flakes of blood or milky in contrast. Besides, it was colored greenish, yellowish or blackish according to the causative agent that was in accordance to Özkan et al. (2011). Other clinical signs as dullness, pale mucous membranes with sunken eyes due to excessive water and electrolytes loss, depression with lethargy and inappetence (Ghanem et al., 2012; Constable et al., 2016).

The severity of clinical signs according to the degree of infection and persistent diarrhea; as in the current study mild clinical signs showed reluctant to move , elevated body temperature, complete anorexia, high respiratory rate, low pulse rate and delayed skin fold test time which agreed with(Constable et al., 2016). Increasing of respiratory rate and decreasing pulse rate occurs due to stimulation of nervous system by the increased viscosity of the blood due to excessive water loss accompanied with minerals disturbance (hyponatremia and hypochloremia coupled to hypermagnesemia, hyperphosphatemia and hyperkaliemia) (Özkan et al., 2011).

While sever clinical signs were observed prior to death in the two reported cases that showed complete recumbency sternally/laterally, in ability to stand, move or suckling, profuse watery yellowish fouled odor diarrhea, severe dehydration (prolonged skin fold tent and pale white mucous membranes), labored breath, hypothermia and bradycardia which agreed with (Simon, 2018).The diarrheic calves suffered from not only GIT disturbances, dehydration, electrolyte loss and acidosis; but also impaired the functions of many systems or organs as lungs, kidneys and liver (Constable et al., 2016).

Regarding to serum biochemical findings the observed changes in the levels of serum Na and K could be due to

excessive water loss with faces which leads to dehydration and impaired cell membrane permeability (Seifi et al., 2006; Ghanem et al., 2012). Serum urea and creatinine concentrations were elevated in the diarrheic calves that might be due to deficit in renal blood perfusion thus reducing urine formation and alteration in renal function as hyponatremia, hypochloremia and hyperkalemia as previously reported by Singh et al. (2014).

Chronic inflammation of GIT and pathological affection of liver (Chernecky, 2013) might be the cause of recorded elevation of serum AST, ALT and GGT diarrheic calves. On the other hand, serum glucose, Albumin and total protein levels were diminished at the diarrheic calves due to the excretion of those parameters in the intestinal lumen with diarrhea that coincided with Constable et al. (2016). Moreover, the significant increase of serum triacylglycerols that was in accordance to Bozukluhan et al. (2017) due to the impaired liver functions and lipolysis of fatty tissue with impairment of fatty acids synthesis during inflammation and infections.

Cortisol is the main hormone released in case of stress to restoring homeostasis and physiological conditions. In the current study there was increase in cortisol in diarrheic calves to relieve stress resulted from the clinical and biochemical disturbances caused by diarrhea (Fujiwara et al., 1996).

CPK enzyme plays a significant role in energy homeostasis of tissue cells and ensures a constant ATP level in the cells; so higher plasma concentration of CPK associated with tissue damage, poor muscular tissue reperfusion, hypoxia, fatigue and increased permeability of muscles membrane following stress and sudden metabolism changes. So, it is useful for evaluation of disorders involving damage to the myocardium, skeletal muscle and central nervous system. So, its increase in diarrheic calves indicates fatigue, metabolic disorders and skeletal muscle degenerations (Minka et al., 2010).

CRP is a diagnostic inflammatory biomarker which rises rapidly in case of inflammation or tissue destruction; so, it used for monitoring the health and vitality as it binds with the metabolites released from cellular degeneration to re-enter the host metabolic processes to not be utilized by pathogen. Therefore, CRP increase in diarrheic calves indicates tissue damage and pathogenic infection (Tóthová et al., 2013).

5. CONCULOSIONS

Calf diarrhea may have a negative health impact by changing the clinical picture and biochemical blood parameters in Egyptian buffalo calves. Consequently, a special care should be given toward the buffalo calves less than three months old to avoid risk factors associated with calf diarrhea.

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