Some Biochemical and bacteriological (aerobic bacterial isolates) studies on milk fed calves suffering from diarrhea and tympany

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Abstract
Forty milk fed calves (5-30days) of both sexes were subjected to the clinical, biochemical and bacteriological investigation. The calves were divided into two groups; the first group involved those calves showed the clinical sings (diarrhea and tympany). The second group was clinically free and was apparently healthy (served as control group). The observed clinical sings of the first group were: depression, weakness some cases suffered from recumbence and varying degree of dehyderation and diarrhoea. The biochemical examination revealed increase metabolic acidosis associated with normochloremia, significance decrease in biocarbonate, hyperkalemia, hyponatremia , reduced mean values of partial Co_2 (PCo_2) and partial O_2 , (PO_2), elevated values of packed cell volume, and urea as a negative result to the dehydration. The bacteriological examination revealed that *E. coli* was isolated in incidence (42.5%), Salmonella species (12.5%) and *Staphylococcus aureus* (16.6%). *E.coli* isolates were identified serologically into the following serogroups : 4, 5, 2 and 2 strains respectively,(O119), (O101), (O26), (O55) while 4 strains were untype, in an incidence of salmonella isolates were serologically identified as 2 *Salmonella typhimurium* and 3 Salmonella enteritidis. In vitro sensitivity pattern of isolated strains proved that Enrofloxacin, Gentamycin and chloramphenicol were the most effective drugs for the isolates.

Key words: *Salmonella typhimurium, dehyderation, milk fed calves.*

Introduction

Proper calf rearing is the rational starting point for economic beef and dairy production. A complete understanding of its multifactorial aspects is of paramount importance. Calf scour is a broad descriptive term referring to diarrhea in calves. Calf scour is not a specific disease, but is actually a clinical sign of a disease complex with many possible causes **Costello, (2002); De Visser and Breukink, (1984).** Diarrhea in farm animals, especially in neonatal calves is one the most challenging clinical syndromes encountered by practicing large animals veterinary practitioners throughout the word **Mailk *et al.*, (2013).**

Calves do best under consistent circumstances, sudden changes of these circumstances, test their ability to cope and may adversely affect the health status of the animals. Scour is one of the most common and costly diseased conditions that can affect the newborn calves, and accounts for high morbidity and mortality rates resulting in tremendous economic losses in beef and dairy industries **Barrington *et al.*, (2002)** Several disease problems include chronic tympanitis, chronic vomiting, cachexia, unthriftiness, abomasal erosions and ulcers are documented in veal calves associated with improper adaptation to the changes in the husbandry. Some of these problems are associated with the failure of the reticular groove reflex, resulting in ruminal drinking **Breukink *et al.*, (1988).** Persistent ruminal drinking causes a syndrome characterized clinically by a variety

of symptoms including unthriftiness or lethargy, growth retardation, inappetence, recurrent tympany, abdominal distension and diarrhea with soft and clay-like feaces **De-Visser and Breukink (1984) and Breukink *et al.*, (1988).**

Also, dysfunction of the esophageal groove reflex resulted in ruminal drinking, neonatal diarrhea, rumen acidosis, dyskeratosis of the ruminal mucosa with fatal outcome **Dirr and Dirksen, (1989).**

Anion gap is an index that can be used to determine the possible causes of metabolic acidosis. The mathematical difference between sodium (Na^+) and potassium (K^+) as the major cations and the sum of chloride (Cl^-) and bicarbonate (HCO_3) as the major anions, represents the anion gap **Malley, (1990).** Meanwhile, clay -like or too-light faeces may have been resulted from either increased resorption of water in colon **Breukink *et al.*, (1988),** or increased hydrolysis of fat within the rumen with decreased intestinal absorption that affect the consistency and color of faeces **Stocker *et al.*, (1999),**

it is caused by a combination of many risk factors. Interaction between environments, nutritional imbalance, improper colostrum intake and virulence of pathogens provoke the imbalance of intestinal equilibrium resulting in diarrhoea **Radostits, *et al*., (1994).**

Meanwhile, severe ruminal acidosis in young calves has resulted in systemic acid-base disturbances with resultant metabolic acidosis **Gentile *et al.*, (1998).**

Diarrhoea is considered to represent a problem that may lead to great economical losses as it consider the most important cause of calves morbidity and mortality especially among newly born calves in Egypt and allover the word **Byomi, *et al.*, (1996).** Several bacterial species may be involved in diarrhoea, the most important being is certain strains of *E.coli*, Salmonella species, and Campylobacter species **Harbby, (2002).**

Aim of the work

The aim of the present study is to investigate and throw the light on the biochemical changes accompained bacterial infection in diarrhetic milk fed calves.

Materials and Methods

Animals.

Forty calves of both sexes, aged between 5 and 30 days were involved in this study. The calves were classified into 2 groups. Calves of group one (n=30) showed the clinical signs diarrhea, abdominal distension and rough coat. Calves of group No. 2 (n=10) were apparently and clinical healthy and were used as control group.

Samples and sampling protocol. All samples were collected from private farm at El-Dakahlia Province.

(A) Blood samples and Biochemical examination. Three types of blood samples were obtained from each calf by jugular vein puncture.

The first blood samples were obtained in hiparinized tube and were used for determination of PCV (**Coles, 1986).**

The second blood samples were obtained in specialized syringes with rubber plugs and were used for determination of blood pH, $PC0_2$, PO_2 and bicarbonate concentrations. Blood gas measurements and acid-base determination were carried out using blood gas analyses (ABL- Radiometer) according to **Malley (1990).**

The third blood samples were obtained in non heparinized tubes for obtaining blood serum and were used for biochemical analysis of the selected parameters, particularly chloride (Cl^-), sodium (Na^+) potassium (k^+), blood urea nitrogen (BUN) and creatinine (Cr). The biochemical analysis of samples were carried out spectrophotometrically using, the commercial test kits according to the methods described by **Feldkamp (1974)**, **Varley *et al.*, (1980) and Faweett (1960) respectively.**

It is well documented that the induction of the esophageal groove reflex in the milk-fed calves depends on certain prerequisites **Dirr and Dirksen, (1989).**

Persistent ruminal drinking, causes a syndrome characterized clinically by a variety of symptoms including unthriftiness or lethargy,

growth retardation, inappetence, recurrent tympany, abdominal distension, dry coat and diarrhea with soft and clay-like feaces. **(De-Visser and Breukink 1984 Breukink *et al.* 1988).**

(B) Feacal sample and Bacteriological examination.

A total of forty rectal, feacal samples were collected from (30 each of diarrhoeic calves and 10 each of apparently healthy calves) Samples were obtained by means of sterile probes introduced into the rectum of each calf, and kept in sterile plastic bottle. All samples were labeled, kept in ice box and sent to the laboratory as quickly as possible for bacteriological examination. The samples were collected from private farm at El-Dakahlia Province.

I- Bacteriological examination.

a- Media.

Rappaporte-Vasiliadis broth (R.V., Oxoid, CM 669), Trypticase soya broth

Blood agar; MacConky's agar (Oxoid, CM 7); Eosin methylene blue agar (EMB, Oxoid CM 69); Xy lose lysine deoxycholate agar (XLD, Oxoid, CM469)

b- Methods. Isolation and identification of bacterial agents.

Each feacal sample was divided into 3 portions under aseptic condition. The first part was streaked directly onto predried surface of Blood agar; MacConky's agar; Eosin methylene blue agar; Xylose lysine deoxycholate agar. The plates were incubated aerobically at 37°c for 24 hours. The second faecal part was inculated into Rappaporte--Vasiliadis broth and incubated at 43°C /24 hours, loopfules from incubated R.V. broth were streaked onto XLD agar plate with incubation at 37°c for 24 hours.

The growing colonies on various plates were examined morphologically, culturally and biochemically according to **Edwards and Ewing, (1972); Finegold and Baron (1986) ; Collins, *et al*. (1995) and Quinn, *et al.* (2002).**

- The identified *E.coli* strains were tested for enterotoxin production through growing the *E.coli* isolate in trypticase soya broth at 37 °C in stationary culture over night, culture were centrifuged at 4000 r.p.m. for 20 minutes. The supernatant was tested using commercially VET-RPLA kits (reversed passive Latex agglutination, Oxoid, TD 0920A) following the manufacturer's direction & Scotland, *et al.* (1989).

II- Serological identification.

1-E.coli.

Serological identification of purified *E.coli* strains using available agglutinating Coli test sera (Behring Werk, AG Marburg) was done according to manufacturer's instruction. (Labn, Germany).

2- Salmonella.

The biochemically identified Salmonella strains were subjected for serological identification as described by **Edwards and Ewing, (1972)**; **Kaufmann (1973)** and the instruction of the manufacturer (Denken Selken Co. LTD, Tokyo, Japan).

III- In vitro antibiotic sensitivity test.

The disc diffusion technique was performed on the isolated bacteria using Muller-Hinton media (Oxoid). Ten chemotherapeutic disks kindly supplied by Oxoid and namely Ampicillin, Amoxycillin, Chloramnphenicol, Enrofloxacin, Erythromycin, Gentamycin, Streptomycin, Penicillin. Oxytetracycline and Trimethoprim-sulphamethoxazole were used. The degree of sensitivity was interpreted according to **Koneman *et al*., (1994) ; Quinn, *et al.,* (1994) and Oxoid Manual,(1998).**

Statistical analysis

Statistical analysis of variance (ANOVA) and t -test was carried out following the method described by **Kirkwood (1989).**

Results

Table (1). Hematological and biochemical findings in healthy and diarrheic calves.

Parameters items	PCV %	BUN Mg|dl	creatine mg\dl	pH value	HCO_3 ppm	PCO_2 ppm	PO_2 ppm
control	31.5[a] ±1.4	11.7[a] ±0.9	1.11[a] ±0.26	7.21[a] ±0.62	21.11[b] ±0.92	42.93[a] ±1.8	31.2[a] ±1.6
diarrheic calves	38.73[b] ±3.65	27.51[c] ±1.75	2.6[a] ±0.55	6.69[a] ±0.31	10.58[a] ±0.35	39.12[a] ±0.93	29.6[a] ±1.3

Means with different letters in the same raw are significantly different at P< 0.05

Table (2). Blood serum macro and micro element levels (mean ± SE) in healthy and diarrheic calves.

Parameters items	Sodium Na+ mmol/L	Potassium K+ mmol/L	Chloride Cl	Copper Cu µg/L	Iron Fe µg/L	Phosphorus P mg/dl	Zinc Zn µg/L	Calcium Ca mg/dl
Control	134.9[b] ±2.71	5.2[a] ±0.19	105.9[a] ±4.2	64.80[b] ±2.55	160.12[b] ±3.32	6.21[a] ±0.16	169.95[c] ±3.22	10.11[a] ±0.21
diarrheic calves	128.34[a] ±2.66	4.95[a] ±0.23	105.3[a] ±3.1	40.95[a] ±1.62	125.81[a] ±3.10	4.42[a] ±13	110.82[a] ±2.64	8.13[a] ±0.11

Means with different letters in the same raw are significantly different at P< 0.05

Table (3). Incidence of bacteria isolated from the examined calves.

Bacterial isolates	Condition of calves				Total	
	Diarrhoeic calves		Apparently healthy			
	30		10			
	N	%	N	%	N	%
E.coli	16	53.3	1	10	17	42.5
Salmonella spp.	5	16.6	-	-	-	12.5
Staph. aureus	3	10	2	20	5	12.5

Table (4). Serological identification of isolated *E.coli* and Salmonella.

Source of samples	*E.coli*									
	O_{119}		O_{101}		O_{26}		O_{55}		UNTYPE	
	N	%	N	%	N	%	N	%	N	%
Diarrhoeic calves	4	23.52	5	24.41	2	11.76	2	11.76	4	23.52
Source of Samples	Salmonella									
	S. typhimurium				S. enteritides				Untype	
	N		%		N		%		N	%
Diarrheic Calves	2		40		3		60		-	-

Table (5). In vitro sensitivity test for the isolates from diarrheic calves.

	Antibiotic disc	E.coli	Salmonella	Staph.aureus
(1)	Ampicillin 10 µg/L	R*	R*	R*
(2)	Amoxycillin 25 µg/L	R*	R*	R*
(3)	Entrofloxacin 15 µg/L	+++	+++	++
(4)	Gentamycin 10 µg/L	+++	+++	++
(5)	Streptomycin 10 µg/L	R*	R*	+
(6)	Penicillin 10 µg/L	R*	R*	+
(7)	Chloramphenicol130 µg/L	+++	+++	+
(8)	Oxytetracyclin 30 µg/L	++	+	+
(9)	Trimethoprim-Sulpha-Methoxazol 1.25-23.75 µg/L	+	+	R*

+ = Degree of sensitivity
* R = Resist

Discussion

Although the rumen is not fully functional in the new born calves the reticulorumen and esophageal groove are involved in the digestive processes with consequent disease condition **Dirr and Dirksen, (1989)**. The results of this study revealed an increase in metabolic acidosis hyponatremia in diarrhoetic calves, hyperkalemia and normochloremia, reduced blood pH and partial CO_2 (PCO_2) as showed in table (1) and (2) these results agree with that obtained by **Gentile *et al*., (1998) and Stocker *et al.*, (1999)** who recorded that an extreme metabolic acidosis, hyponatremia, hyperkalemia and normochloremia. In addition small reduction in PCO_2.

The diseased calves had ruminal acidosis because of accumulation of acidic metabolites such as lactic acid and butyric acid produced by abnormal fermentation within the rumen. These results are in agreement with that obtained by **Breukink *et al.* (1988), Dirr and Dirksen (1989) and Stocker *et al.* (1999).** Retardation of growth in the ruminal drinkers could be attributed to hyper or parakeratosis of the ruminal mucosa and villous atrophy within the small intestine as reported by **Breukink *et al.*, 1988, Vanweeren Keverling Buisman *et al.* (1988), Fisher and McEwan (1997).**

The diarrhoeic calves had ruminal acidosis because accumulation of acidic metabolites such as lactic acid and butyric acids produced by abnormal fermentation, these results are agreed with that obtained by **Stocker *et al*., (1999)**. The results of this study also revealed an increase in PCV, BUN and creatinine which may have been resulted from dehydration with hemoconcentration that lead to a reduced glomerular filtration with oliguria as found by **Stocker *et al*., (1999)**. Our results showed a decreased level of bicarbonate therefore such type of metabolic acidosis may be the result of reduced bicarbonate and accumulation of lactic acid resulting in mixed metabolic acidosis,some finding were noticed by **Omole *et al*., (2011)**. In case of metabolic acidosis the calves attempted to compensate acidosis by elimination of CO_2 in the lungs as indicated by reduction in POC_2 and HCO_3, these results agree with **Stocker *et al*., (1999)**. The increasing potassium level in the blood obtained in our study have reduced the myocardial potassium that

interferes with depolarization and cause bradycardia and varying degrees of heart block with eventual death of the diseased calves and these finding agreed with **Fisher and Mc Ewan, (1997)**. Regarding to PO_2 values showed slightly decrease as showed in table (1) diarrhea is very common in the calves and can have an impact both economically and in terms of animal welfare. The bacteriological examination of 40 feacal samples from calves revealed (67.5%) positive bacterial infection. Results in Table (2) revealed that the isolated bacterial pathogens were *E.coli* (42.5%), Salmonella spp. (12.5%) and Staphylococcus aureus (12.5%). Nearly similar pathogens were isolated by **Mc Donough, *et al.,* (1994)**. *E.coli* is a gram negative lactose fermenting, indole positive facultative anaerobe of human and animal intestinal flora. Generally in *E.coli* infections, diarrhoea occurs through the effect of enterotoxins which stimulate adenyl cyclase activity of intestinal and capillary epithelium (heat labile toxin, LT), resulting in hypersecretion of electrolytes (Na^+ & Hco_3) and an increased diffusion of water into lumen of the intestine **(Kaske, 1993)**

Salmonella spp. infection causes severe mucosal damage and increase permeability of the mucosal epithelium that result in uncontrolled lakage of water and ions into the intestinal lumen **(Robinsion and Haxtable, 1988)**.

In this study *E.coli* was the most prevalent isolates with an incidence of (42.5%). All the isolated *E.coli* strains from diarrhoeic calves were enterotoxigenic produce heat labile enterotoxin (LT) when tested by VET-RPLA kits and serologically identified as 23.52% (O_{119}); 24.41% (O_{101}); 11.76% (O_{26}); 11.76% (O_{55}) and 23.52% untype strains, as showed in table (4). These results are agreed with **Harrby, (2002)** who recorded that *E.coli* is the main cause of diarrhea affecting newly born calves. On sterotyping of salmonella organism from examined samples (40%) were recognized as salmonella typhimurium and (60%) as salmonella enteritides these results were agree with **Novert and Hammad (2001)**. Staphylococcus aureus were isolated from the examined samples from (12.5%) similar results were obtained by **Manaa *et al*., (1993)**. In vitro sensitivity testing of isolates revealed that most isolates were highly sensitive to enrofloxacin, Gentamycin and Chloramphenicol and resistance to Ampicillin and Amoxycillin (table 5). Nearly similar results were reported by **Maarouf and Mobark (2007)** .Finally we can conclude that cases of calves diarrhea is a sequel of infection with various intestinal pathogens which is exaggerated with stress factors such as improper feeding, unsanitary condition of drinking and bad hygienic surroundings. Managemental procedures are very important in controlling the disease showing any potential zoonotic risks and aiming at the reduction of losses among the new born calves and we must put in consideration the adverse effect of this disease on blood gas and acid-bas status.

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