

Bacteriological And Biochemical Studies On Diarrhea In Neo-Nate Calves In Sharkia Governorate

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ABSTRACT

The present investigation was planned to investigate 41 diseased calves aged 14-40 days old weighting 45-60 kg in a private farm at El-Sharkia-Province. The calves suffered from anorexia, depression, elevation in body temperature and diarrhoea with offensive odors, mucoid discharge and varying degrees of dehydration.

Entropathogenic E. coli, Klebsiella sp. and Proteus sp. were isolated and identified from rectal samples of diseased calves in percentage of 24.4 %, 22.0 %, 17.1 % and mixed infection (E.Coli and proteus sp of 19.5%, and E.Coli and Klebsiella sp.) of 14.6%.

Also cryptosporidium sp. was found in faecal samples, either alone (7.7 %) or mixed with E.coli bacteria (5.8 %), with Proteus sp. (3.8 %) and with Klebsiella sp (5.8 %) and incriminated as the causative agent of diarrhoeic neonatal calves.

Blood of diarrhoeic calves caused by bacteria and/or crytosporidia showed significant increase in erythrocytic count, haemoglobin content, packed cell volume percent and total leukocytic count.

The serum biochemical study presented a significant elevation in the transaminases enzymes (AST-ALT), alkaline phosphatase, urea, creatinine and potassium but significant decrease in glucose, total protein, inorganic phosphorus, magnesium and sodium.

Antibiogram studies showed that the gentamycin was the highest effective than other tested antibiotics .Excellent improvement in clinical health status, blood and serum constituents were observed post treatment with fluid therapy (Rehy- dro zinc) and gentamycin injection and sulphamix either alone or together according to the causes of the diarrhoea in cattle calves.

INTRODUCTION

Calf diarrhoea is a multifactorial disease entity that can have serious financial and animal welfare implications in both dairy and beef suckler herds. It has been estimated that 75% of early calf mortality in dairy herds is caused by acute diarrhoea in the pre-weaning period (1).

Diarrhoea in calves has been associated with a variety of aetiological agents including viral scour pathogens(2), enterotoxigenic E. coli (ETEC),Environmental challenges in the form of inclement weather and diet changes have also been associated with ETEC infections (3), Protozoa (4), nutritional factors acting together with infectious agents (5)

The purpose of this investigation is to:

- 1-Determine and identify the prevalence of the major contagious causes of diarrhea.
- 2-The isolated bacterial strains were tested for *in vitro*-sensitivity
- 3-Observation and record alterations in some haematological and biochemical constituents of calves with diarrhea,
- 4- As well as, trials for the treatment of diarrhea .

MATERIAL AND METHODS

During the period between sept.2007to Feb.2008 this study was carried out on 41 calves suffering from diarrhoea aged from 14-40 days old weighting 45-60 kg in a private farm at El-Sharkia-Province.

Sampling

Faecal samples

Individual faecal samples were collected from all examined animals using sterile probes introduced into the rectum and kept in sterile plastic bottles. All samples were labeled and examined parasitologically by;

a-Direct fecal smear (6).

b-Concentration flotation technique (7).

c-Thin faecal smear were made and left to dry, then fixed with methanol for 10 minutes, and stained with modified Ziehl-Neelsen stain (8). Finally, the smears were screened under the oil immersion lens for detection of cryptosporidial oocysts.

Faecal swabs

Were taken within 10-20 hours of the onset of diarrhea before any treatment for bacteriological examination.

Bacteriological examination

Sterilized faecal swabs were taken and incubated on nutrient broth at 37°C for 24h., then subcultured into selective media (9), followed by bacterial isolation and identification (10).

In vitro- antibiotic sensitivity test

Using the disk agar diffusion method (11) in accordance with the instruction of the antibiotic disk supplier (Oxoid).

Blood samples and biochemical studies

Two blood samples were collected from control and each diseased calf through jugular vein puncture before and after treatment by 10, 20 and 30 days post treatment. First sample was collected in heparinized tube for haematological examination (12).

The second blood samples were collected without anticoagulant to obtain clear, non haemolysed sera for clinico-biochemical study of serum aspartate aminotransferase* (AST), alanine aminotransferase* (ALT) (13), alkaline phosphatase (14), total protein (15), glucose (16), urea (17), creatinine (18), sodium, potassium and chloride by flame photometer (Corning Model 410) (19), calcium (20), inorganic phosphorus (21) and magnesium (22).

Drugs

Gentamycin (garavet) R was obtained as a bottle contain 100ml and each 1 ml contain 50mg gentamicin sulphate as pharmaceutical preparation from Memphis Company for pharmaceutical and chemicals company, Egypt

- sulphamix

- Fluid therapy (Rehy- dro zinc).

Statistical analysis

Test for significance was made by calculating student (T) Table (23).

RESULTS AND DISCUSSION

Age of susceptibility and clinical signs

Diarrhoea was reported in calves up to 40 days old and this may be due to that the immune system of animals at young age is not well developed and the maternal immunity would not withstand variable infections these results were in coincidence with those supported in sheep and goat (24).

The clinically diseased calves suffered from inappetance, do not drink or suckle, with gastrointestinal discomfort, depression, elevation in body temperature, with varying degrees of dehydration, diarrhoea which varied from soft to profuse watery diarrhoea with offensive odors and mucoid discharge similar to those of cryptosporidiosis (25).

Bacteriological examination

Table 1 showed that the bacteria responsible for diarrhea in calves were *Escherichia coli* (*E.coli*) of 10 calves with a percentage of (24.4%), Other species of family Enterogenic bacteriaeae as *Kelbisella* sp and *proteus* sp. were also detected as *Kelbisella* sp 9 (22.0 %), and *Proteus* sp 7 (17.1 %), The higher incidence of various serotypes of *E.coli* from diarrhoeic calves supports the idea of the higher susceptibility of this age to infection with *E.coli*. (26,27), this may be due to inadequate transfer of passive immunity from the dam which considered the main risk factor for colibacillosis.

The mixed infection with other species of family enterobacteriaceae were recorded, as *Proteus* sp. and *E.coli* 8 (19.5 %), *Kelbisella* sp. and *E.coli* 6 (14.6 %), these were nearly similar to that recorded in kits, lambs and calves (28).

Table 1. Causes and Percentages of diarrhea in examined newly born calves

Etiological Agent	Type of microorganisms	No of samples (52)	%
Bacterial	E.coli	10	24.4
	Klebsiella sp.	9	22.0
	Proteus sp	7	17.1
Mixed bacterial	Proteus sp. and E.coli	8	19.5
	Klebsiella sp. and E.coli	6	14.6
Parasite	Cryptosporidium sp.	4	7.7
Mixed Bacterial and parasite	Cryptosporidium sp. & E.coli	3	5.8
	Klebsiella sp. & Cryptosporidium sp.	3	5.8
	Cryptosporidium sp. & Proteus sp.	2	3.8

Antibacterial sensitivity tests

Disc diffusion test *in vitro* against previous isolated bacteria from diarrhoeic calves either in alone or mixed infection revealed that gentamycin was the highest effective on all isolated organisms than other tested antibiotics, followed by flumouquine, enrofloxacin, neomycine and erythromycin. But all isolated microorganisms were resistance to colistin and Nalidixic acid as in Table 2. Tested serogroups of E.coli isolated from diarrhoeic calves were found to be sensitive to gentamycin and enrofloxacin and resistant to oxytetracycline, penicillin G and nalidixic acid (29)..

parasitological examination

As shown in Table 1 cryptosporidium sp. is the six important cause of diarrhoea among examined calves, Cryptosporidium sp. was found alone in 4calves (9.8%) or mixed with E.coli 3 (7.3 %), with Klebsiella sp. 3 (7.3 %) and Proteus sp 2 (4.9 %), these attributed to young age animals are severely affected by Cryptosporidium, as far as they are immunologically immature and consequently have a greater susceptibility to the infection by this parasite, (30).

Only 4 calves (9.8%) which were suffering from severe weakness, sever watery diarrhoea and were positive for both bacterial infection, and cryptosporidium sp. had been died. Mixed infection are frequently seen and clinical signs are usually more severe where more than pathogen is involved (1). This mortality could

be attributed to fluid loss, electrolytes imbalance, malabsorption and protein losing enteropathy, (31).

Haematological results

The hematological finding in all diarrhoeic calves due to bacteria or cryptosporidia either alone or mixed infection were illustrated in Tables 3, showed significant increase in RBCs. counts, haemoglobin, packed cell volume percent M.C.V and total leukocytic count. This may be attributed to the occurrence of dehydration and hemoconcentration arised from diarrhoea (32). The increase in W.B.Cs may reflect the condition of bacterial enteritis which may be by primary or secondary to parasitic infestation or could be attributed to the destructive effective of cryptosporidia oocysts on the epithelial cells of the gastrointestinal tract walls (33).

Biochemical results

It so clear evident from that diseased calves suffered from diarrhoea due to bacteria or cryptosporidia either alone or mixed infection induce significant elevation in the transferase enzymes (AST-ALT)and alkaline phosphatase indicated the presence of marked hepatic damage This may be attributed to degenerative changes and necrotic processes accompanied the formation of intestinal and hepatic lesions due to bacterial infections and its toxins (34). Changes in liver enzyme activity due to cryptosporidium could be attributed to the epithelial tissues damage of the intestinal walls by the parasites and its toxins (35).

Table 2. Sensitivity tests of isolated organisms against different antimicrobial agent.

Antibiotic	E.coli	Proteus sp.	Klebsiella sp.	E.coli & Proteus sp.	Proteus sp. & Klebsiella sp.	Klebsiella sp. & E. coli
Gentamycin	+++ S	++ S	++ S	++ S	++ S	++ S
Enrofloxacin	S +++	+ S	+ S	S+	+ S	S++
Flumequin	S++	+ S	+ + S	++ S	R	++ S
Neomycin	S +	++ S	R	+ S	R	+ S
Nalidic acid	+ S	R	R	+ S	R	+ S
Colistin	R	R	R	R	R	R
Erythromycin	S ++	+++ S	++ S	+ S	+ + S	++ S

S=sensitive R=resistance

Table 3. Haemogram of clinically normal and diarrhoeic calves due to bacteria or cryptosporidia either alone or mixed infection before and after treatment

parameter	Healthy	Bacterial					Cryptosporidia				Mixed infection			
		Pre treatment	Post treatment(days)			Pre treatment	Post treatment(days)			Pre treatment	Post treatment(days)			
			10	20	30		10	20	30		10	20	30	
RBCs 10 ⁶ /c.mm	7.07± 0.41	9.76 ± 0.44**	8.46 ± 0.27*	8.13 ± 0.76	7.09± 0.33	9.08± 0.57*	8.66 ± 0.38*	8.07 ±0.62	7.76 ± 0.73	9.99± 0.59**	9.4 ± 0.75 *	8.65 ± 0.34*	7.87± 0.55	
HB g m %	7.76 ± 0.24	9.89 ± 0.34**	9.65 ± 0.48**	9.04 ± 0.51*	7.96 ± 0.87	10.01 ± 0.69*	9.62± 0.58*	8.88 ± 0.87	7.89 ± 0.76	10.36 ± 0.74**	9.78 ± 0.45 *	9.06 ± 0.84	8.07 ± 0.77	
PCV %	26.87 ± 1.06	35.98 ± 0.67***	33.89 ± 1.76**	30.78 ± 0.99*	28.64 ± 1.82	33.45 ± 2.11**	29.57± 0.69*	29.01 ± 1.77	27.89± 1.11	34.55 ± 1.16**	32.06± 1.58*	30.67 ± 1.98	28.89 ± 1.11	
leukocytes (10 ³ /cmm)	8.90 ± 0.78	10.25 ± 0.19*	9.96 ± 0.10	9.18 ± 0.58	8.79 ± 0.47	9.92± 0.17*	9.99 ± 0.54	8.95 ± 0.45	8.64 ± 0.48	10.67 ± 0.31*	10.02 ± 0.15*	9.87 ± 0.45	8.65 ± 0.65	

*significant at P < 0.05 ** Significant at P < 0.01

Serum urea and creatinine changes showed an elevated levels in all diarrhoeic calves Table 4. During dehydration, there is an elevated serum urea and creatinine (36, 37). Such findings may be attributed to excessive production of urea and creatinine by increased protein catabolism processes in severe toxic and febrile conditions, reduced renal function and the beginning of nephropathological changes (38).

It was noticed that the diarrhoea in calves due to bacteria or cryptosporidia either alone or mixed infection induce significant decrease in total protein, similar to that reported in buffalo-calves (33). The present observation in diarrhoeic calves due to bacterial cause may be attributed to the general unthriftiness which may affect worsely the hepatic parenchyma resulting in the failure of the liver for protein synthesis.

About cryptosporidia infestation it could be attributed to the inability of the gut in parasitized animals to absorb and assimilate the haemopoietic principals Regarding blood serum total protein, albumin and globulin and a state of anorexia and inability of the synthesis proteins (39).

Regardless of cause, the disease is characterized by development of dehydration and electrolyte abnormalities which are believed to be responsible for many of the clinical signs and for the frequent high mortality (40- 42). Table 4 showed that significant increase in serum potassium, may be due to potassium leave the intracellular space to the extracellular one instead of hydrogen ion to compensates acidosis occurred during diarrhoea (43). This can attributed to the presence of bacterial and parasitic agents

together and their effect on health condition of calves. These results are in agreement with the finding obtained in neonatal calves (44).

Table 5 showed significant decrease of sodium, magnesium, calcium and inorganic phosphorus, of all diarrhoic calves due to bacterial, cryptosporidia and mixed infection, similar results were recorded (45) who reported that sodium & chloride are particularly exposed to loss in diarrhoea stools as they are components of the gastrointestinal secretions, or Hypophosphatemia and hypomagnesemia mainly due to decrease in feed intake and mal absorption (43).

Treatment and efficacy

Treatment the calves that suffered from diarrhoea under this study with gentamycin injection, electrolytes and/or sulphamix with previously mentioned doses revealed that the cure rate was 95.1%, while 2 calves died at a ratio of 4.9% at day 6 of treatment. Scouring calf loses large amounts of fluids and electrolytes, such as sodium and chlorine. Usually the cause of death in scouring calves is dehydration and acidosis, or increased body acidity although antibiotics are commonly the first treatment cattlemen administer for scours;

fluids are the most important therapy to correct dehydration.

In conclusion, the occurrence of diarrhoea in calves is a result of the complex interactions of three sets of factors: the calf and the dam, the calf's environment, including management, and infectious agents specially *E.coli* and cryptosporidium infection must be considered in diarrhoic calves less than one month of age, where, they cause haematological and biochemical alterations, Whatever the microbial cause of scours, the most effective treatment for a scouring calf is rehydration by administering fluids, which returned to the normal levels by use of balanced electrolyte solution, gentamycin and sulphamix either alone or together.

So preventing Scours by, Heifer Management, Cow Management and Clean Calving Environment. Strict hygienic measures and effective control for contagious pathogens should be applied.

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Table 4. Mean value of some biochemical parameter of clinically normal and clinical diarrhoic calves due to bacteria or cryptosporidia either alone or mixed infection before and after treatment.

parameter	healthy	Bacterial					Cryptosporidia					Mixed infection				
		Pre treatment	Post treatment(days)			Pre treatment	Post treatment(days)			Pre treatment	Post treatment(days)					
			10	20	30		10	20	30		10	20	30			
AST (U/L)	58.33 ± 2.45	74.11 ± 4.88**	69.78 ± 3.32*	66.49 ± 2.78*	60.23 ± 4.34	72.45 ± 1.45***	69.23 ± 3.43*	64.12 ± 2.33	59.12 ± 1.11	82.55 ± 3.45***	77.06 ± 4.44**	70.56 ± 2.53*	63.32 ± 3.19			
ALT (U/L)	29.02 ± 1.21	40.23 ± 2.76**	38.98 ± 2.74*	32.98 ± 2.22	30.77 ± 2.09	42.65 ± 2.96**	39.43 ± 2.87**	35.45 ± 1.56	31.65 ± 2.56	44.29 ± 2.45***	42.76 ± 3.65**	38.43 ± 2.83*	32.46 ± 1.49			
Alk. Ph (U/ml)	79.08 ± 3.98	83.43 ± 3.65*	83.43 ± 3.45	82.43 ± 1.98	80.56 ± 2.37	90.56 ± 3.93*	88.39 ± 3.43*	84.69 ± 2.87	81.39 ± 1.45	94.87 ± 3.74**	90.87 ± 3.98*	86.01 ± 2.54	82.67 ± 3.43			
Urea (mg/dl)	22.99 ± 1.56	35.72 ± 2.97**	34.56 ± 2.76*	30.45 ± 2.34	24.45 ± 1.56	33.89 ± 2.65**	29.32 ± 1.75*	26.04 ± 1.92	24.97 ± 2.43	36.39 ± 2.76**	33.59 ± 2.62*	29.28 ± 2.49*	27.03 ± 1.58			
Creatinine (mg/dl)	1.34 ± 0.36	2.53 ± 0.35*	2.36 ± 0.26*	2.06 ± 0.33	1.49 ± 0.23	2.67 ± 0.14**	2.35 ± 0.24*	1.94 ± 0.34	1.29 ± 0.45	2.73 ± 0.16**	2.55 ± 0.28*	2.04 ± 0.87	1.54 ± 0.83			
Glucose (mg/dl)	73.08 ± 2.67	52.09 ± 2.65***	53.76 ± 3.76**	66.45 ± 1.11*	71.76 ± 2.54	52.45 ± 2.34***	58.34 ± 2.18**	63.36 ± 1.65*	68.45 ± 2.45	50.56 ± 2.25***	53.56 ± 2.23**	59.76 ± 3.08*	66.78 ± 1.34			
Total Protein (g/dl)	8.44 ± 0.45	6.05 ± 0.55**	6.77 ± 0.72*	7.87 ± 0.17	8.23 ± 0.34	6.72 ± 0.21*	6.79 ± 0.11*	6.82 ± 0.79	7.90 ± 0.90	6.09 ± 0.46**	6.98 ± 0.37*	7.08 ± 0.27*	7.12 ± 0.54			

*insignificant at P < 0.05 ** Significant at P < 0.01 *** Significant at P < 0.001

Table 5. Mean value of serum electrolytes of clinically normal and clinical diarrhoic calves due to bacteria or cryptosporidia either alone or mixed infection before and after treatment.

parameter	healthy	Bacterial					Cryptosporidia				Mixed infection			
		Pre treatment	Post treatment(days)			Pre treatment	Post treatment(days)			Pre treatment	Post treatment(days)			
			10	20	30		10	20	30		10	20	30	
Calcium (mg/dl)	9.11 ± 0.78	6.10 ± 0.81*	7.34 ± 0.27*	7.75 ± 0.59	8.87 ± 0.90	7.12 ± 0.47*	8.12 ± 1.11	8.72 ± 1.21	8.98 ± 0.98	5.98 ± 0.64*	6.43 ± 0.34*	7.26 ± 0.96	8.28 ± 0.98	
Inorganic phosphorus (mg/dl)	4.81 ± 0.78	2.67 ± 0.12*	3.57 ± 0.13*	3.45 ± 0.39	4.66 ± 0.83	2.55 ± 0.20*	3.05 ± 0.19*	4.04 ± 0.91	4.79 ± 0.95	2.86 ± 0.18*	2.99 ± 0.24*	3.59 ± 0.66	3.99 ± 0.41	
Magnesium (mg/dl)	2.66 ± 0.43	1.43 ± 0.17**	1.76 ± 0.23*	2.07 ± 0.67	2.23 ± 0.47	1.39 ± 0.25*	1.95 ± 0.33*	2.18 ± 0.47	2.54 ± 0.48	1.19 ± 0.25*	1.58 ± 0.28*	2.03 ± 0.28	2.38 ± 0.26	
sodium (mEq/L)	138.39 ± 4.79	112.64 ± 2.41**	119.71 ± 3.04**	125.74 ± 3.91*	131.90 ± 4.52	117.74 ± 2.24**	121.52 ± 2.91**	126.92 ± 3.84*	129.93 ± 4.28	105.93 ± 3.83***	117.94 ± 2.92**	123.18 ± 3.54*	125.76 ± 5.83	
potassium (mEq/L)	4.84 ± 0.56	6.94 ± 0.58**	6.67 ± 0.53*	5.89 ± 0.93	4.69 ± 0.85	6.59 ± 1.27**	6.12 ± 0.43*	5.94 ± 0.98	5.03 ± 0.87	7.17 ± 0.18*	6.87 ± 0.67*	6.69 ± 0.53*	5.95 ± 0.94	

*insignificant at P < 0.05

** Significant at P < 0.01

*** Significant at P < 0.001

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المخلص العربي

دراسات بكتريولوجية وبيوكيميائية على الإسهال في العجول حديثة الولادة بمحافظة الشرقية

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أجريت هذه الدراسة على ٤١ رأساً من العجول الرضاعة عند عمر ١٤-٤٠ يوم بإحدى المزارع بمحافظة الشرقية وكانت تعاني من فقد الشهية، ارتفاع بدرجة الحرارة، إسهال بدرجات متفاوتة مما أدى لحدوث حالات من الجفاف وقد أجريت هذه الدراسة لمعرفة مسببات الإسهال في العجول حديثة الولادة وتأثيرها على صورة الدم وبعض الوظائف البيوكيميائية.

وبالفحص البكتريولوجي أمكن عزل البكتريا المسببة للإسهال: ميكروب القولون العصوي، ميكروب بروتيس، ميكروب كلبيلا، كما وجدت العدوى البكتيرية المشتركة (الميكروب العصوي مع ميكروب بروتيس - الميكروب العصوي مع ميكروب كلبيلا).

وفي محاولة لعلاج هذه الحالات تم عمل اختبار الحساسية لهذه المعزولات البكتيرية ودلت النتائج أن الجنتاميسين أكثر المضادات الحيوية تأثيراً على هذه المعزولات.

وبالفحص الطفيلي لمسببات الإسهال تبين وجود طفيل الكريبتوسبوريدا سواء منفرداً أو عدوى مشتركة مع البكتريا (ميكروب القولون العصوي - ميكروب بروتيس وميكروب كلبيلا).

وبدراسة التغيرات في سيرم العجول المصابة بالإسهال الناتج عن الإصابة بالبكتريا أو طفيل الكريبتوسبوريدا أو العدوى المشتركة بينهما وجدت زيادة معنوية في أنزيمي الترانس فيريز (AST) (ALT) الفوسفاتيز القاعدي، اليوريا، الكرياتينين والبوتاسيوم ونقص معنوي في مستوى الجلوكوز، البروتين الكلي، الفوسفور، الماغنسيوم و الصوديوم

ودلت هذه الدراسة حدوث التحسن الملموس واختفاء الأعراض الإكلينيكية وعودة وظائف الكبد والكلى وصورة وقياسات الدم لوضعها الطبيعي للعجول المصابة بالإسهال بعد تعويضها أولاً بما فقدت من السوائل والأملاح المعدنية جراء الإسهال بالمشاركة مع حقن الجنتاميسين للعدوى البكتيرية وحقن سلفامكس للعدوى الطفيلية (الكريبتوسبوروديوم) حال وجودهما.