

**SOME STUDIES ON CLINICAL, HEMATOLOGICAL
AND BIOCHEMICAL CHANGES IN DIARRHOEIC
NEONATAL BUFFALO CALVES WITH
REFERENCE TO HYGIENIC
CONDITIONS**
(With 4 Tables)

By

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**بعض الدراسات عن التغيرات الاكلينيكية والدموية والبيوكيميائية لحالات الاسهال
فى العجول حديثة الولادة بالنسبة للظروف الصحية**

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أجريت هذه الدراسة بمزرعة كلية الطب البيطرى- جامعة قناة السويس على عدد ٢٨ من عجول الجاموس التى تعاني من فقدان الشهيه ، ارتفاع فى درجة الحرارة ، درجات مختلفة من الجفاف الناتج عن الاسهال ذو الرائحة الكريهه مع وجود مخاط مدمم بالبراز ، تكونت المجموعة الضابطة من ١٠ عجول أصحاء. تم عزل وتصنيف الميكروب القولونى، السلمونيلا والسودوموناس من عينات البراز او مسحات بالمستقيم للعجول المصابه ، زيادة على ذلك تبين بالفحص البكتريولوجى لعدة اماكن من ملاعب العجول واحواض المياه ان هناك سيتروباكتر، بروتيس والميكروب القولونى، الكاجين بيكالس، شيجلا، سودوموناس والكليسيلا بنسب مختلفة. بالدراسة المعملية على عينات الدم للعجول السليمة صحيا والمريضة تبين ان هناك زيادة معنوية فى كلا من كرات الدم الحمراء والبيضاء وحجم الخلايا المضغوطة وتركيز الهيموجلوبين فى مجموعة العجول المريضة عن المجموعة الضابطة. بالتحليل الكيمىائى الحيوى لمصل العجول المريضة تبين ان هناك انخفاض معنوى فى مستوى البروتين الكلى، الجلوكوز، الصوديوم والكلوريد. بينما ارتفع البوتاسيوم معنويا عن المجموعة الضابطة. هذا وقد حدث تحسن ملموس فى الاغراض الخارجية وصورة الدم ومكونات المصل للعجول المريضة وذلك بالعلاج بالامبسيلين ٢٠٪ مع العلاج باستخدام السوائل وذلك جنبا الى جنب مع الاجراءات الصحية.

SUMMARY

This study was carried out on 28 diseased buffalo calves suffered from anorexia, elevation in body temperature, depression with varying degrees of dehydration and diarrhoea with offensive odour and bloody mucoid discharge. Other ten clinically healthy buffalo calves served as control group were also included. These calves were belonging to the farm of faculty of Veterinary Medicine, Suez Canal University. Enteropathogenic *E. coli* (12 isolates), *Salmonella* sp. (5 isolates) and *Pseudomonas* sp. (8 isolates) were recovered and identified from diseased rectal samples and incriminated as the causative agent of diarrhoeic neonatal calves. Bacteriological examination of different locations of calve's yard soil and water troughs was carried. This examination revealed the presence of *E. coli*, *Proteus* sp., *Citrobacter* sp., *Klebsiella* sp., *Pseudomonas* sp., *Shigella* sp., and *Alcaligenes feacalis* in different percentages. The hematological study was conducted on blood samples of apparently healthy and diseased calves showed significant increase in both total erythrocytic and leucocytic count, packed cell volume and haemoglobin concentration in diarrhoeic calves versus to the control ones. Blood serum biochemical analysis revealed significant decrease in the level of total protein, glucose, sodium and chloride, while blood serum potassium level was significantly increased in diarrhoeic calves than in control ones. Excellent improvement in clinical symptoms, blood picture and blood serum constituents were observed following treatment with ampicillin 20% in combination with fluid therapy and application of good sanitary measures.

Key words: Biochemical changes-diarrhoeic buffalo-calves.

INTRODUCTION

Diarrhoea is still one of the most important causes of calf mortality and morbidity that lead to severe economic losses. The incidence of diarrhoeal diseases in neonatal calves increased in the recent years depending on the sanitary and hygienic managements (*ABD EL-HAMID, 1977*).

Regarding the most common basic causes of diarrhoea, a lot of work had been done of which enterotoxogenic *E. coli*, *Salmonella* species and *Clostridium* (*TENNANT et al., 1972; ABS EL-HAMID, 1977; AMER et al., 1985; SNODGRASS et al., 1986 and FARID et al., 1992*).

Diarrhoea in young pre-weaned calves causes remarkable disturbances in clinical signs and blood parameters (*BLOOD et al.*, 1983; *HASSAEN et al.*, 1983; *HASSEN et al.*, 1985 and *MOTTELIB et al.*, 1992).

The undue use of antibiotics in veterinary medicine constitutes a public health (hazard by development of super-resistant bacteria (*BERCHNEIDER & ARGENZIO*, 1983).

The present investigation was designed to study the aetiological agents causing diarrhoea in neonatal buffalo-calves and their sensitivity to various antibiotics. Moreover, the study included some haematological and biochemical alterations in response to enteric diseases as well as to determine the effectiveness of specific antibacterial agents. Also the role of water and soil hygiene in developing and existence of diarrhoeic agents was considered.

MATERIAL and METHODS

This work was carried out on 38 buffalo-calves belonging to the Farm of Faculty of Veterinary Medicine, Suez Canal University. Among these animals, 28 calves showed signs of diarrhoea, while 10 clinically normal ones served as control. The age of these buffalo-calves ranged between 2-4 weeks, and their weights varied from 35-44 kg. The animals were kept free in an open yard, watered freely, and fed on natural suckling. Both diseased and control buffalo-calves were subjected to clinical examination.

From each animal, two rectal faecal samples were collected before and at the seventh day after treatment. The first sample was taken directly from the rectum using sterile swab for bacteriological examination (*WILSON & MILES*, 1984), while the second faecal sample was taken in plastic bag for parasitological studies (*COLES*, 1980).

The antibiotic sensitivity test of different isolated microorganisms against various antibacterial agents was carried out using disc method reported by *GOULD & BOWIE* (1952).

Two blood samples were collected from both control and diseased groups just before seven days post treatment. The first blood sample was taken in tubes containing anticoagulant (heparin concentration of 0.1 mg/ml. blood) used for determination of both total erythrocytic and leucocytic counts per mm³, packed cell volume (*SCHALM*, 1975) and haemoglobin (*VAN-KAMPEN*, 1961). The second blood sample was used for obtaining clear serum to determine total protein, glucose, and chloride according to *King and Wotton* (1959) and *WERNER et al.* (1970) respectively. Sodium and

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potassium levels were analysed on a flame photometer according to the method of *OSER (1965)*.

Forty soil samples each of 100 grams were collected from calves' yard in sterile screw-capped bottles. Twenty samples of them were collected from different areas of the yard already exposed to sun-rays, while the other twenty soil samples were collected from areas under yards shed. All soil samples were examined physically according to *CRUICKSHANK et al. (1975)* to determine pH and moisture content. The temperature of soil samples was recorded at time of sampling using Beckmann thermometer. Meanwhile, bacteriological examination of these samples was done after *MERCHANT & PACKAR (1977)*.

From watering trough, 50 water samples were obtained for physical analysis to determine pH and temperature at time of sampling. Bacteriological examination were also done (*EDWARD and EWING, 1972*).

The diseased calves were isolated and watered separately from special buckets which were thoroughly washed and disinfected before use. The superficial layer of 10 cm yard's soil was removed and replaced by new uncontaminated soil as well as periodical continuous turn-over with Quick lime (2 tons/acre) was tried.

Chemotherapeutic trial was done by using Ampicillin 20% at a dose rate of 20mg/kg B.w./12 hours (in the form of powder dissolved in 10 ml of distilled water and given orally using drenching gun) for five successive days. Moreover, oral fluid replacement therapy according to *BLOOD et al., (1983)* at rate of 100-ml/kg body weight in eight divided doses was tried as follows:-
Oral electrolyte formula (*BLOOD et al., 1983*).

Sodium chloride,	117 g.
Potassium chloride,	150 g.
Sodium bicarbonate,	168 g.

Potassium phosphate,	135 g.
Total	570 g.

For 1000 ml of oral solution, add 5.7 g. of dry mixture, to which also 50 g. of glucose was added.

The obtained data were statistically analysed according to *SNEDECOR and COCHRAN (1969)*.

RESULTS

The clinical signs observed on the diseased buffalo-calves from diarrhoea revealed that most of the cases showed loss of appetite, fever (40.7- 41.8°C) that decreased just after the onset of diarrhoea, depression, increase in pulse and respiratory rates profuse diarrhoea with offensive odour accompanied with mucoid and/or bloody discharge. The hind-limbs were splashed with faeces and also varying degrees of dehydration were observed.

The results of bacteriological examination of rectal faecal swabs obtained from apparently normal buffalo-calves as well as the diseased ones are shown in table (1). *E. coli* was the most important organism incriminated as the causative agent of diarrhoea in pre-weaned calves. *E. coli* which showed agglutination with anti K99 serum standard was considered as enteropathogenic *E. coli*. The other obtained faecal bacterial isolates were represented by *Salmonella* sp. and *Pseudomonas* sp.

As shown table (2) revealed that the causative bacterial isolates were sensitive mainly to ampicillin 20%. On the other hand, parasitological examination of faecal samples revealed negative results.

The results of physical and bacteriological examinations of soil samples from calves, yard under different circumstances as well as water samples are shown in table (3). The physical examination of soil revealed that high moisture content (5.0-5.7%), pH value (7.0-7.5) and temperature (37.5-38°C) in shadow locations, while in sunny ones moisture content, pH and temperature were 1.0-1.5%, 6.7 & 38.5-39, respectively. Bacteriological examination of soil (under shadow) and water samples revealed that most isolates related to family Enterobacteriaceae.

Results of blood examination (RBCs, WBCs, Hb and PCV) of clinical normal calves and those suffering from diarrhoea just before and seven days after the onset of treatment (Table. 4) revealed highly significant ($P < 0.001$) increase in erythrocytic count, packed cell volume and total leucocytic count. Also a significant ($P < 0.01$) increase in haemoglobin concentration in diarrhoeic calves in comparison with apparently healthy ones. Regarding serum biochemical analysis, (Table. 5) the obtained results showed a highly significant ($P < 0.001$) increase in total protein, a highly significant ($P < 0.001$) decrease in serum glucose, sodium and chloride levels with a significant ($P < 0.05$) increase in serum potassium level in diarrhoeic calves against control group.

The clinical response was noticed on the treated calves at the second to third day of treatment, while good clinical improvement was completely

observed at the fourth to fifth day of treatment, while seven days post treatment haemoglobin and biochemical values were within normal values.

DISCUSSION

The most important cause of economic losses in beef industries is the infectious neonatal diarrhoea (BLOOD *et al.*, 1983). Bacteriological examination of diseased calves in our study revealed that the enteropathogenic *E. coli* was the most prevalent organism representing 66.6%, *Salmonella* sp. 27.7% and *Pseudomonas* sp. 44.4%. These results agreed with the findings of TZIPORI, (1981) and FARID (1992). The production of enterotoxin by *E. coli* stimulates mucosal adenylyl cyclase activity that leads to increase cyclic adenosine monophosphate (AMP) which in turn increases the intestinal fluid secretion from the systemic circulation resulting in varying degree of dehydration, electrolyte imbalance and acidosis (BLOOD *et al.*, 1983). Moreover, *Salmonella* sp. infection causes severe mucosal damage and increases permeability of the mucosal epithelium that result in uncontrolled leakage of water and ions into the intestinal lumen (ROBINSON & HUXTABLE, 1988). BLOOD *et al.* (1983) elucidated that sporadic cases of bovine salmonellosis may occur when animals are exposed to stress.

Physical examination of soil samples were taken from sunny locations revealed lower levels of moisture content, pH and temperature than that of shadow locations. These results coincided with those of ABD EL-KARIM, (1968), ABD EL-HAMID & ZAKI, (1972) and ABD EL-KARIM *et al.*, (1977) who attributed these variations to adverse environmental conditions.

Bacteriological findings of soil samples collected from the under shed were *E. coli* 100%, *Proteus* sp. 70%, *Citobacter* sp. 23%, *Kellebsiella* sp. 10%, *Pseudomonas* sp. 11%, and *Shigella* sp. 12% and *Alcaligenes faecalis* 12%, while from sunny locations were 70, 20, 8, 9, 6 and 10%, respectively). The obtained data reflected the lack of hygienic measures in such yard and agreed with those of ABD EL-KARIM (1968).

Bacteriological examination of water samples showed the presence of family Enterobacteriaceae. The presence of such family in water is considered as an index of undesirable pollution as well as an indicator of faecal pollution (W.H.O. 1985), Isolation of *E. coli* 75%, *Proteus* sp. 62%, *Citrobacter* sp. 40%, *Kellbsiella* sp. 73%, *Pseudomonas* sp. 12% and *Shigella* sp. 14% from samples of water troughs was in agreement with MOUSTAFA *et al.*, (1977) and EL-OLEMY *et al.*, (1989).

Bacteriological examination of faecal swabs, soil and water samples revealed that the most predominant micro-organism responsible for the presence of diarrhoea in buffalo calves was the enteropathogenic *E. coli*. There is relationship between the environmental temp. R.H. and the incidence of those enteropathogenic *E. coli*. This result coincided with that reported by *DEBORAH*, (1972).

Haematological picture is widely used in veterinary medicine to evaluate the general health condition, diagnosis of some diseases (*FREEDLLAND and NAMER*, 1970). In diarrhoeic calves there was a highly significant ($P < 0.001$) increase in erythrocytic count and packed cell volume ($10.206 \pm 0.205 \times 10^6/\text{mm}^3$ & $34.833 \pm 0.439\%$) when compared with apparently healthy ones ($7.806 \pm 0.124 \times 10^6/\text{mm}^3$ & $27.90 \pm 0.537\%$), respectively. A significant ($P < 0.01$) increase in haemoglobin concentration was observed in diseased animals ($12.288 \pm 0.237 \text{ gm}\%$) against control ones ($10.450 \pm 0.215 \text{ gm}\%$). These elevations in haemogram values were due to loss of body fluid caused by diarrhoea (*BLOOD et al.*, 1983). Moreover, highly significant increase ($P < 0.001$) leucocytosis was observed in diseased calves ($14.166 \pm 0.240 \times 10^3/\text{mm}^3$) versus to ($8.172 \pm 0.192 \times 10^3/\text{mm}^3$) in apparently healthy ones. This finding coincided with *DALTON et al.*, 1965; *AMER et al.*, 1985 and *MOTTELIB et al.*, 1992. The marked increase in leucocytic count is due to bacterial infections and inflammatory lesions which acted promptly causing greater rise in total leucocytic count and more production of mature and immature neutrophils (*DOXEY*, 1983).

Blood serum biochemical analysis revealed a highly significant ($P < 0.001$) increase in total proteins in diarrhoeic calves ($7.555 \pm 0.065 \text{ mg}\%$) in comparison with clinically normal ones ($6.950 \pm 0.076 \text{ mg}\%$). The hyperproteinemia in calves with enteritis due to *E.coli* and *Salmonella* infection may be due to the excessive loss of body fluids and concentration of some blood constituents especially in case of dehydration (*MANAA et al.*, 1993).

Highly significant ($P < 0.001$) decrease in the level of glucose ($47.555 \pm 0.573 \text{ mg}\%$) in serum of diarrhoeic calves compared with clinically healthy ones ($70.40 \pm 1.087 \text{ mg}\%$) was observed. This result is in close agreement with that reported by *MOTTELIB* (1972) and *Hassan et al.*, (1985) who attributed hypoglycaemia in *Escherichia coli* enteritis in buffalo calves to the alteration in tissue metabolism caused by decreased blood flow and oxygenation, while *COLES*, (1980) elucidated that hypoglycemia in case of enteritis may be due to lack in intestinal absorption.

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The obtained result showed a highly significant decrease ($P < 0.001$) of serum sodium (126.833 ± 0.927 mEq/L) and chloride (87.22 ± 0.946 mg/L) in diarrhoeic calves in comparison with clinically normal ones (139.310 ± 0.735 mEq/L & 100.40 ± 0.98 mg/L, respectively). Our findings coincided with that obtained by *HASSAN et al.* (1985) and *RAGAB et al.*, (1986), who attributed hyponatremia and hypochloremia in diarrhoeic calves to direct loss of sodium and chloride ion via faeces. On the other hand, potassium level in serum of diseased buffalo calves (5.355 ± 0.152 mEq/L) increased significantly ($P < 0.05$) than its level in apparently normal ones (4.90 ± 0.53 mEq/L). This finding was in agreement with *ROUSSAL*, (1983), *HASSAN et al.*, (1985) and *RAGAB et al.*, (1986). Diarrhoea is a common cause of metabolic acidosis due to direct loss of bicarbonate via faeces (*LEWIS & PHILLIPS*, 1972 & *RAGAB et al.*, 1986) and therefore, during diarrhoea the increase of hydrogen ions are buffered by intracellular and extracellular buffers. In exchange for the intracellular movement of H^+ , K^+ enters the extracellular compartment predisposing to hyperkalemia (*RABINSON & HUXTABLE*, 1988).

About chemotherapeutic treatment noticeable clinical improvements were observed following administration of specific antibacterial agent in addition to fluid therapy on the third to fourth day of treatment accompanied with good hygienic measures in soil and water troughs. Moreover, all the disturbed blood parameters were corrected and returned nearly to normal levels at the seventh day of treatment.

From this study, it could be concluded that bacterial calf diarrhoea is responsible for causing remarkable disturbances in haematological and biochemical values through direct and indirect effects due to impaired defence mechanisms. This by turn reflects the disturbance in general health condition of the affected calves. So attention must be paid for careful early clinical and laboratory diagnosis of diseased calves followed by the use of specific antibiotics accompanied by fluid replacement therapy. Finally, and as it is always said, prevention is better than cure, good management of calves from time of birth and until weaning is the key factor in protection them during this critical period of their lives.

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Table (1): Shows the number and percentages of the organisms isolated from fecal swabs of apparently normal and diarrhoeic calves.

Type of micro-organism	10 apparently normal calves		18 diseased calves		Total No. of isolates
	No. of isolates	%	No. of isolates	%	
- Non pathogenic E. coli	8	80	5	27.7	13
- Pathogenic E. coli	-	-	12	66.6	12
- Salmonella sp.	-	-	5	27.7	5
- Pseudomonas sp.	4	40	8	44.4	12
Total number of isolates	12		30		42

Table (2): Shows results of sensitivity tests of isolates against different antimicrobial agents

Therapeutic agents of antibiotic disc.	Concentration of disc	E. coli	Salmonella spp.	Pseudomonas spp.
Tetracyclen	30 µg	(-)	(+)	(+)
Penicilline G.	10 IU	++	+	+++
Ampicilline	10 µg	+++	+++	+++
Colistin	10 µg	++	+	++
Neomycin	30 µg	+	++	++
Erythromycin	15 µg	++	(-)	(-)

Table (3): Examination of soil and water samples from calves yard under different environmental conditions.

Type of sample	No. of sample	Sunlight						Shadow			
		Physical Exam			Bacteriological Exam			Physical Exam			Bacteriological Exam
		PH.	Moist cont. %	Temp.	Percentage of Bacterial isolates %	PH.	Moist cont. %	Temp.	Percentage of Bacterial isolates %		
Soil samples	40	6.7-7.0	1.0-1.5	38.5-39	E. coli 70% Proteus sp. 50% Citrobacter sp. 20% Klebsiella sp. 8% Pseudomonas sp. 9% Shigella sp. 6% Alcaligenes feacalis 10%	7.0-7.5	5.0-5.7	37.5-38	E. coli 100% Proteus sp. 70% Citrobacter sp. 23% Klebsiella sp. 10% Pseudomonas sp. 11% Shigella sp. 12% Alcaligenes feacalis 12%		
Water samples	50					6.8-7.3		37.5-38	E. coli 75% Proteus sp. 60% Citrobacter sp. 40% Klebsiella sp. 60% Pseudomonas sp. 73% Shigella sp. 12% Alcaligenes feacalis 14%		

Table (4): Erythrocytic & leucocytic count; haemoglobin concentration and packed cell volume in apparently normal and diseased calves (before and after treatment).

Condition of calves	No.	RBCS ($10^6/\text{mm}^3$)	WBCS ($10^3/\text{mm}^3$)	Hb (gm %)	PCU %
Apparently normal	10	7.806 ± 0.124	8.172 ± 0.192	10.450 ± 0.215	27.9 ± 0.537
Diseased calves before treatment	18	10.206** ± 0.205	14.166** ± 0.240	12.288* ± 0.237	34.833** ± 0.439
Diseased calves after treatment	18	8.017 ± 0.098	8.742 ± 0.264	10.705 ± 0.203	28.44 ± 0.353

* Significant at ($P < 0.01$)

** Highly Significant at ($P < 0.01$)