

Animal Health Research Institute.

Assiut Laboratory.

Director Prof. Dr. S.M. Nashed.

SOME MICROBIAL AND BLOOD BIOCHEMICAL STUDIES ON BUFFALO CALVES SUFFERING FROM ENTERITIS

(With 4 Tables)

By

A.M. MANAA; A.M. SAYED; A. EL-R-THABET,
and A.M. ABD EL-FATTAH.

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بعض الدراسات الميكروبيولوجية والتغيرات البيوكيميائية في دم عجول الجاموس المصابة بالالتهابات المعوية

أحمد مناع ، أحمد سيد ، محمد الراضى ثابت
أحمد عبد الفتاح

أجريت هذه الدراسة على عدد ٤٠ من عجول الجاموس المصابة بالالتهابات المعوية و عدد ٢٠ من العجول السليمة. هذا وقد تم عزل الميكروب القولوني المعدي بنسبة ٨٠% أما ميكروب السالمونيلا فتم عزله من ٢٠% من الحالات المصابة. بالإضافة إلى أنه قد تم عزل ميكروبات السيدوموناس أيروجينوزا والشيجلا وفطر الكانديدا أليكانس كمسببات للعدوى مع الميكروب القولوني والسالمونيلا بنسبة ١٥% - ٥% - ٢٠% على الترتيب. ولقد تم اختيار حساسية الميكروبات المعزولة لعدد من المضادات الحيوية ووجد أن أكثرهم تأثيراً أميكان - كلورامفينكول - جاراميسين - نيتلا ميسين - ريمكتان وتوبراميسين. شملت الدراسة أيضاً قياس مستوى سكر الجلوكوز والبروتين الكلى فى مصل الدم وتبين وجود انخفاض ملحوظ فى نسبة سكر الجلوكوز فى أمصال العجول المصابة بالتهابات المعوية بالإضافة إلى وجود زيادة ملحوظة فى تركيز البروتين الكلى.

SUMMARY

This study was carried out on 40 diseased buffaloe calves suffered from severe enteritis and 20 clinically healthy ones which served as control group. Enteropathogenic *E. coli* ($O_{70} : k_{80} : O_{114} : B_{16} : O_{26} : B_6$) and *Salmonella* formed the main bacterial causative agents, they were isolated and identified from 80% and 20% of diseased rectal swab samples respectively. *Pseudomonas aeruginosa*, *Shigella*, and *Candida albicans* were also found in form of mixed infection with enterotoxigenic *E. coli* and *Salmonella*. They were isolated from 15%, 5%, and 20% of diseased rectal swab samples respectively. Antibiogram for the bacterial isolates revealed the best sensitive antibiotics were amikain, chloramphenicol, garamycin, netilimicin, rimactan, and tobramycin. In diseased cases blood sera biochemical analysis showed highly significant ($p < 0.01$) decrease in serum glucose level with significant ($p < 0.05$) increase in total proteins concentration.

INTRODUCTION

Enteritis in young calves is a syndrome of great aetiological complexity. In addition to the influence of varied environmental, managemental, nutritional and physiological factors, the infectious agents capable of causing enteritis in the neonatal calves are numerous (SNODGRASS, et al., 1986). The authors added Several reports on the occurrence of individual microorganism as a cause of enteritis are common.

Several studies were carried out elsewhere generally showed that the most important bacterial infectious agents are enterotoxigenic *E. coli* and *Salmonella* species (MOTTELIB, 1972, and MOERMAN, et al., 1982).

E. coli while being a normal gastrointestinal inhabitant, it is also associated with a variety of pathological conditions in animals (SMITH, 1965). Many surveys of calf mortality incriminated *E. coli* as a major cause of death due to "white scour", (SHERWOOD, et al., 1983)

AMER, et al., (1985) and HASSAAN, et al., (1985), detected the predominance of enteropathogenic *E. coli* as a main cause in the pathogenesis of enteritis in calves at Upper Egypt. *Salmonella* species are considered mainly as primarily enteric

organisms in case of enteritis in cow calves (JOHNSTON, et al., 1976 and SNODGRASS, et al., 1986). MOTTELIB, (1972), isolater also *Salmonella* SPP. from buffalo calves suffering from enteritis.

Candida albicans has been recently investigated as an important cause of enteritis in calves (SZIGE and NAGY, 1990).

Many diseased conditions of which digestive disturbances are of great importance, induce sever changes in serum glucose and total proteins levels which reflect the status of animal body (CORNLIUS and KANEKO, 1963). MOTTELIB, (1972) found hypoglycaemia and increase in serum total protein in buffalo calves with enteritis caused by *E.coli* and *Salmonella*. The Same findings were also recorded by HASSAN, et al., (1985) in enteric calves caused by *E.coli*.

The purpose of this study is two folds, firstly, to find out the real bacteriological and fungal causative agents responsible for enteritis in newly born buffaloe calves found in farms located at Assiut Governorate. Secondly to study the effect of enteritis on the blood serum glucose and total protein levels.

MATERIAL AND METHODS

Animals:

Atotal of 60 buffaloe calves were used in this study choosen from farms located at Assiut Governorate. Those 40 calves showed severe entritis, and 20 were clinically healthy and served as control. Clinical signs were recorded. The mean age of calves was 2-6 weeks and the mean duration of enteritis was 3-10 days.

Samples:

Blood samples for biochemical and rectal swabs for bacteriological studies were taken before administration of any treatment and within 12-18 hours of the onset of enteritis.

Bacteriological and biochemical examinations:

Therectal swabs were inoculated aseptically into nutrient broth incubated at 37 C° for 24 hours. and then subcultured into the following media: (Difeco) nutrient agar, MacConky agar, SS agar, XLD agar, as well as Sabouroud's agar.

The isolates were identified according to the colonial morphology, the pigment production, microscopically by Gram stain, and biochemically according to BAILY and SCOTT, (1974). The isolated *Candida* were identified according to their morphological and microscopical appearance (MONICA, 1985).

Serological characterization of isolated strains of *E. coli* were done.

Antibiotic sensitivity tests for bacterial isolates were done using antibiotic discs (Biomerieux) of amoxicilline (25 µg) ampicilline (10 µg), amikacin (30 µg), chloramphenicol (30 µg), colistine (10 µg), erythromycin (15 µg), garamycin (30 µg), nalidixic acid (30 µg), kanamycin (30 µg), netilmicin (30 µg), rimactan (30 µg), tetracycline (30 µg) and tobramycin (30 µg).

Blood samples were withdrawn from the jugular Vein. Blood serum was separated immediately. Serum glucose and total protein levels were estimated using test kits supplied from Boehringer Mennhein (GERMANY). Statistical analysis of obtained Serum biochemical data was performed according to method of KALTON (1967).

RESULTS

The most prominent clinical signs of diseased calves were loss of appetite, fever (40.5 °C) which was decreased just after the onset of diarrhoea, depression, increased pulse and respiratory rates, profuse diarrhoea with varying degrees of dehydration. The faeces was watery, sometimes bad with foetid odour and containing mucous and/or blood.

Microbiological isolates, antibiogram study, range, mean values and standard deviations of serum glucose and total protein were demonstrated in Table 1, 3 and 4 respectively.

Serological characterization of the strains of *E. coli* isolated were shown in Table (2).

DISCUSSION

Among buffalo calves the enteric infections predominates and cause severe economic losses to producers from reduced live weight, inefficient feed conversions, delay marketing, deaths; costly preventive and therapeutic programs (SAYED, 1988).

The results recorded in table 1 revealed that the enteropathogenic *E. coli* ($O_{70} : k_{80} : B_{16}, O_{26} : B_6$) were the main microbiological cause of enteric infections in diseased buffalo calves. (Table 1, 2). This results agree with that previously reported by MOTTELIB, (1972), AMER, et al., (1985), and SNODGRASS, et al., (1986). The mechanism by which the organisms may produce the disease is the toxins production. These toxins may be entro or endotoxins. The enterotoxin is produced by entro pathogenic *E. coli* proliferating in the anterior small intestine causing hypersecretion through

intestinal epithelium, some pathological changes in the jejunum and ileum, and diarrhoea. Endotoxin is thought to act either directly or indirectly by means of an anaphylactic reaction, which could produce diarrhoea, various forms of enteritis and oedema disease (SOJKA, 1971). However there are some enteropathogenic *E.coli* which do not produce toxin, but they adhere to the surface of the enterocytes of the large intestine, and the affected calves pass bright red blood in diarrhetic faeces (HALL, 1985).

In this study salmonella infection formed the second main cause of enteritis in diseased buffaloe calves (Table 1). Several outbreaks of salmonellosis in newly born calves were reported previously by many authors, (TUTT, et al., 1974; JOHNSTON, et al., 1976, and CLEGG, et al., 1983). The main clinical manifestations of salmonellosis were loss of appetite, depression, dullness, fever, and diarrhoea. The faeces was watery, having a putrified smell and containing mucous and clots of blood (MOTTELIB, 1972). The main pathological lesion was that of deep inflammation of the ileum (JOHNSTON, et al., 1976).

Shigella, *Pseudomonas aeruginosa*, and *Candida albicans* were also isolated (Table 1). They were mainly presented in the form of mixed infections either with *E.coli* or *salmonella* infection. In this respect our results concerning candida, albicans agreed with SZIGE and NAGY (1990) who recorded that *Candida albicans* is a pathogenic microorganism encountered in intestine especially of calves reared in high stocking rate and bad hygienic conditions.

Antibiogram for the isolates of enterotoxigenic *E.coli*, salmonella and *psudomonas aeruginosa* revealed that the best sensitive antibiotics are amikain, chloramphonical, garamycin, netilimicin, rimactan, and tobramycin (Table 3). This result was previously reported by MONICA, (1985).

Blood serum biochemical analysis revealed a highly significant decrease ($P < 0.01$) in glucose with significant increase ($P < 0.05$) in total protein in buffalo calves suffered from enteritis, when compared with clinically healthy ones. (Table 4). This result was generally supported by MOTTELIB (1972), and HASSAAN, et al., (1985). The obtained hypoglycaemia may be due to the lack of intestinal absorption in case of enteritis (COLES 1967). MOTTELIB, (1972) attributed such decrease in serum glucose level found in buffaloe calves suffering from *E coli* enteritis the alteration in tissue metabolism caused by decrease blood flow and oxygenation.

MICROBIAL, BLOOD BIOCHEMICAL STUDIES CALVES & ENTERITIS

Another explanation had been given by MADISON (1964) which was due to the elevation of the ketone bodies in case of gastero-enteritis, and such elevation in blood ketones led to depression of the blood glucose.

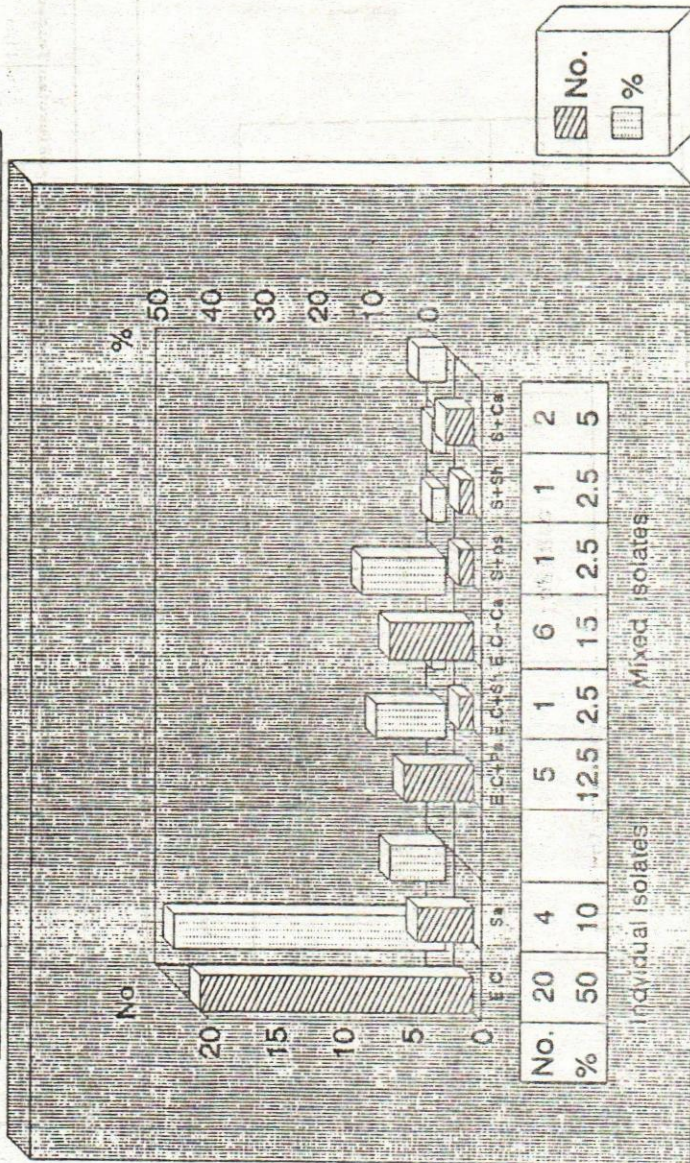
MOTTELIB, (1972), explained the increase of serum total protein level found in calves with enteritis due to *E.coli* and *salmonella* infection may be a sequel of due to the excessive loss of body fluids and concentration of some blood constituents especially in case of dehydration. AMER, *et al*, (1985), confirmed these findings reporting high level of heamatocrite ($40.00 \pm 1.63\%$).

REFERENCES

- Amer, A.A.; Hassaan, N.K.; El-Sebaie, A.; Bayoumi, A.H. and Ibrahim, A.H., (1985): Studies on an outbreak of colibacillosis among Holstein Friesian calves in the New Valley. Egypt: I-clinical, Heamatological, Aetiological and Pathological alterations. Assiut Vet. Med. J. Vol. 14, No. 27: 151-157.
- Baily, W.R. and Scott, B.G. (1974): Diagnostic microbiology, isolation and identification of Pathogenic microorganism. 4 th Ed. The Mosby Comp. Saint Louais.
- Clegg, F.G.; Chiejina, S.N. and Duncan, Al. (1983): Outbreaks of Salmonella newborn infection in dairy herds and their relationship to management and contamination of the environment . Vet. Record 112 (18) 580-584.
- Coles, E.H. (1967): In: Veterinary Clinical Pathology. W.B. Saunders, Philadelphia and London.
- Cornlius, C.E. and Kaneko J.J. (1963): In: Clinical Biochemistry of domestic animals Academic press, New York and London.
- Hall, G.A.; Reynolds, J.; Chanter, N; Morgan, J.H.; Person, K.R.; Debeny, I.G.; Bland, A.P.; Bridgel, J.C. (1985): Dysentery caused by Escherichia Coli (5 102-9) in calves by natural and experimental disease. J. Vet. path. 22(2) 156-163.
- Hassaan, Kn.K.; El-Sebaie, A. and Amer, A.A. (1985): Studies on an outbreak of colibacillosis among newly Holstein Friesian calves in the new valley, Egypt II Some serum biochemical and electrolyte changes. Assiut Vet. Med. J. Vol 14 No. 27: 161-168.
- Johnston, K.G.; Jones, R.T.; Jones, R.T. and Soulsby, E.J. (1976): Salmonellosis in calves due to lactose fermenting *Salmonella typhimurium*. Vet record 98 (3) 276-278.

- Kalton, G. (1967): Introduction to statistical ideas from social scientists. 2nd Ed. Acad. Press. (London.).
- Madison, L.L. (1964): Clinical and experimental action of Ketones on hepatic, glucose output and peripheral glucose utilization. J. Lab. Clin. Med., 63:177.
- Hoerman, A.; Leauw, P.W.; Zidderveld, F.G. and Tiessink, J.W. (1982): 12 th world congress on diseases of cattle. The Netherlands. world Association for Buiatrics p 228:232.
- Monica, C. (1985): Medical laboratory manual for tropical countries. Vol. II: Microbiology. 1st Ed. University Press, Cambridge.
- Mottelilb, I. A. (1972): A study on changes of blood in buffalo calves suffering from enteritis due to different causative agents. Thesis. Fac. Vet. Med. Assiut Univ. Assiut, Egypt.
- Sayed, A.M. (1988): Clinical and some blood trace elements change following respiratory and alimentary disturbances among calves M.V.Sc. Thesis, Fac. of Vet. Med. Assiut Univ., Egypt.
- Sherwood, D.; Snodgrass, D.R.; and Law son, G.H. (1983): Prevalence of enterotoxigenic *E.coli* in calves in Scotland and northern England. Vet. Record. 113 (3): 203-212.
- Smith, H.W. (1965): Observation on the aetiology of neonatal diarrhoea (Scours) in calves J. Pathol. Bacteriol., 84, 117.
- Snodgrass, D.R.; Terzolo, H.R.; Sherwood, D.; Campbell, I.; Menzies, J.D. and Syngé B.A. (1986): Aetiology of diarrhoea in young calves. Vet. record. 119(12) 31-34.
- Solka, W.J. (1971): Enteric disease in new-born calves and lambs due to *E.coli* infection. Vet. Bulletin, 41(7) 509:521.
- Szige, G. and Vagy, B. (1920): Investigation of the pathogenic role of *Candida* and *Foculopsis* isolated from cases of calf pneumonia and diarrhoea. Magyar Allatorvosok Lapja., 15(7): 399-403.
- Tutt, J.B.; Hoare, B.I.; Harthoorn, A.M. (1974): Disease associated with *S typhimurium* in cattle. Vet. record 95(12) 334-337.

Table(1) The isolated microorganisms from infected calves



Total No. = 40

E.C : E. Coli.

PS : Pseudomonas

Ca : Candida

Sa : Salmonella

Sh : Shigalla

Individual isolates

Mixed isolates

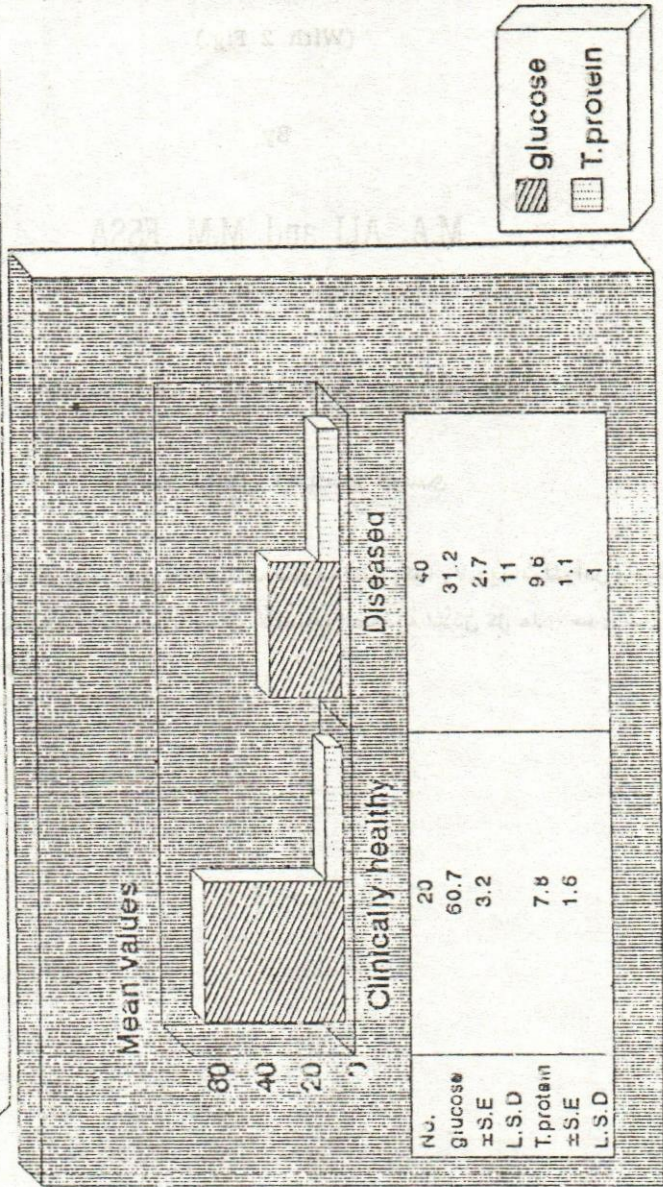
Table (2): Serological characterization of the isolated E.Coli.

No.	Enteropathogenic	Eterotoxogenic	
		Toxogenic	Non Toxogenic
16	O ₇₀ / K ₈₀	- ve	+ ve
7	O ₁₁₄ / B ₁₆	- ve	+ ve
8	O ₁₂₆ / B ₁₆	+ ve	- ve
1	O ₂₆ / B ₆	- ve	+ ve

Table (3): Antibiogram of the isolates of E.Coli, Salmonella and Pseudomonas.

Isolate	Antibiotic Sensitivity												
	Amoxi- lline	Ampicil- iline	Amikain	Chloramp- henicol	Colistine	Erythro- mycin	Garam - ycin	Nalidixic acid	Neomycin	Netill micin	Rimactan	Tetracyc- line	Tobram- ycin
<u>F.Coli</u>	-	+	++	+++	-	+	+++	+	++	+++	+++	+	+++
<u>Salmonella</u>	-	-	++	+++	+	-	++	+	++	+++	+++	+	+++
<u>Pseudomonas</u>	-	-	++	++	-	-	+++	-	+	+++	+++	-	++

Table (4): Mean values, S.D of Serum glucose and total proteins in normal and diseased buffaloe calves.



1: L.S.D <0.05 11: L.S.D <0.01