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HAEMATO-BIOCHEMICAL CHANGES AND ANTIBIOTIC SENSITIVITY TO *ESCHERICHIA COLI* ASSOCIATED WITH CONCURRENT ENTERIC AND SEPTICAEMIC INFECTION IN CALVES

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ABSTRACT

Concurrent enteric and septicaemic *Escherichia coli* infection was recorded in six local calves aged between 8 to 12 months old for the first time in Bangladesh during the period from May to June 2002. Bacteriologic examination of both the faecal and blood samples of six febrile calves showed positive for *E. coli* of all the six faecal (100%) and only two blood (33.33%) samples. Clinical examination revealed significantly ($p < 0.01$) high rectal temperature at peak febrile stage which varied from 103.5 to 106 ^{0}F with a mean of 104.7 ± 0.85 ^{0}F. The fever associated signs and clinical anaemia were recorded in all these calves. Haematological examination showed significantly ($p < 0.01$) decreased haemoglobin (5.58 ± 0.45 g%), packed cell volume (19.83 ± 1.95 %), total erythrocytic count (3.52 ± 0.58 10^6/ mm^3) and total leukocytic count (6.68 ± 0.44 10^3 / mm^3) which indicates anaemia and leukopenia at the peak febrile stage of the disease. Biochemical studies showed significantly ($p < 0.05$) increased that serum magnesium and iron values were at fever stage, whereas serum phosphorus significantly ($p < 0.01$) decreased at the 4th day (convalescent stage) but no significant ($p > 0.05$) change was observed with the serum calcium, sodium, potassium and chloride values between prefever and fever stage. Antibiotic sensitivity pattern of *E. coli* isolated from faecal and blood samples revealed that these isolates were found to be resistant to penicillin, ampicillin and amoxycillin, moderately sensitive to gentamicin and streptomycin but highly sensitive to tetracycline. This indicates that tetracycline could be the drug of choice for both the septicaemic and enteric *E. coli* infection in calves.

Key words: Haematobiochemical, *Escherichia coli*, enteric, septicemic, infection, calves

INTRODUCTION

Colibacillosis is an important disease of newborn farm animals, caused by pathogenic serotypes of *Escherichia coli.* It is a complex disease in which several different risk factors interact with pathogens. The *E. coli* infection in calves is described as either enteric which is characterized by varying degrees of diarrhoea, dehydration, acidosis or septicaemic which is manifested by severe illness, shock and rapid death (Radostits *et al.*, 2000). Review of literature revealed that certain serotypes of *E. coli* cause diarrhoea and others cause septicaemia. The major virulence attributes of the enteric strains of *E. coli* in calves are the K99+ adhesin antigen and the heat-stable enterotoxin, whereas 078 and 31a are invasive and cause septicaemia but K88 related fimbrial antigen occurs on some enteric and septicaemic strains (Contrepois *et al.*, 1986). Although the colibacillosis have been reported to be associated with enteric or septicaemic disease in newborn farm animals elsewhere (Radostits *et al.*, 2000) but *E. coli* infection have been reported to be associated with diarrhoea in young calves in Bangladesh (Debnath *et al.*, 1987 ; Amin *et al.*, 1988 ; Hossain *et al.*, 2002). This paper describes a concurrent enteric and septicaemic *E. coli* infection in young calves with its effects on haemato-biochemical values and antibiotic sensitivity to common antibiotics.

MATERIALS AND METHODS

Six non-descriptive local breed of calves (3 male and 3 female), aged between 8 to 12 months old were purchased from the local market under the 'Calf morbidity and mortality' project No. 99 / 04 / AU on the month of May 2002. These calves were maintained in the Large Animal Experimental House of the Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh which is located adjacent to the BAU Veterinary Clinic. These experimental animals were allowed to graze day time in the uncultivated field adjacent to the animal house and Veterinary clinic. After acclimatization of the calves in the new environment, parasitological examination of faecal samples, rectal temperature and certain haemato-biochemical values were determined of all the six experimental calves. All these calves were affected with stomach fluke disease (paramphistomiasis) and these were successfully treated with Tetranid® (Techno Drugs) ¼th bolus / calf orally.

On the month of June 2002, all the six experimental calves suddenly developed anorexia, fever and mild diarrhoea. The rectal temperature was recorded twice daily at the morning and evening. Faecal samples and venous blood were collected aseptically for bacteriological studies as described by Cowan and Steel (1979).

Haemato-biochemical studies

Blood for haematologic and serum analyses was collected in double-oxalate containing and plain tubes, respectively. Haemoglobin concentration, total erythrocytic count (TEC) and total leukocytic count (TLC) were determined by Spancer Haemoglobinomter and Haemocytometer, respectively, and the packed cell volume (PCV) was measured following centrifugation of Wintrob haematocrit tubes as described by Samad (2001).

The biochemical constituents of serum of calves at different stage of the *E. coli* infection were determined with the help of Spectronic® Genesys™-5 (Spectronic Instrument, Inc., Rochester, NY) as per procedure of the diagnostic kits (Human Gesellschaft fur Biochemica und Diagnostica mbH, Max-Planck-Ring 21, D-65205 Wiesbaden, Germany). Serum calcium was determined with complete test kit Cat. No. : 10011 at 570 nm, phosphorus with the test kit Cat. No. 10027 at 340 nm, magnesium with test kit Cat. No. 10010 at 520 nm, iron with test kit Cat. No. 10229 & 10230 at 623 nm, sodium with test kit Cat. No. 573351 at 410 nm, potassium with test kit No. 10118 at 578 nm and chloride with test kit No. 10115 at 590 nm wavelength as per instructions of the kit manufacturer (Human GMBH, Germany).

***In vitro* sensitivity**

Sensitivity of isolated *E. coli* organisms to antibiotic was studied mostly on blood agar plates using the commercial standardized antibiotic discs (Sanofi Diagnostics Pasteur, 9243 Marnes-La-Coquettes, France) with the antibiotics of penicillin 6 µg, streptomycin 10 iu, ampicillin 10 iu, amoxycillin 25 µg, tetracycline 30 iu and gentamicin 10 iu as per method described by Tripathi and Soni (1982) and Joshi *et al.* (1986). Sensitivity was expressed as 3+, 2+, 1+ and R expressing 'high', 'moderate' 'minimum' (least) and 'resistant' levels of sensitivity, respectively.

Statistical analysis

Results were analyzed statistically with the help of Student 't'- test for significance as described by Gupta (1982).

RESULTS AND DISCUSSION

Six calves, aged between 8 to 12 months were purchased from the local market but detailed information concerning breeding, length of gestation, and medicinal history of the dam and feeding of colostrum to newborn stage was not available. All the six calves suddenly developed fever about one month after procurement.

Bacteriologic culture of faecal samples

Bacteriologic culture of faecal samples of the six calves were conducted and all the faecal samples (100%) were found positive for *Escherichia coli* (Table 1) but no other organism could be isolated from the faecal samples.

Bacteriologic culture of blood samples

E. coli was isolated only from two blood samples (33.33%) of the six tested febrile calves (Table 1). The major portals of entry of *E. coli* initiating bacteriaemia are presented to be the gastrointestinal tract. These observations are in conformity with the earlier reports of Taleb *et al.* (2001) who reported pneumoenteritis in calves, caused by *E. coli* as one of its important cause calf mortality

Clinical findings

All the six calves naturally infected with *E. coli* showed signs of high rectal temperature which varied from 103.5 to 106 ^{0}F with a mean of 104.7 ± 0.85 ^{0}F (Table 1). Variation of body temperature at the different stages of the disease is presented in Table 1. Other signs included weakness, lethargy, decreased intake of food, mild diarrhoea and dehydration and pale conjunctival mucosa. These observations support the report of Aldridge *et al.* (1993) who reported neonatal septicaemia in calves caused by *E. coli.*

Haematologic findings

Haematological values especially haemoglobulin (Hb), packed cell volume (PCV), total erythrocytic count (TEC) and total leukocytic count (TLC) were determined at the different stages of the disease like pre-fever, fever, convalescent and recovered stages are presented in Table 1 and Fig. 1. The mean values of Hb, PCV, TEC and TLC were found significantly ($p < 0.01$) reduced at the peak febrile stage in comparison to pre-fever and recovered stage of the diseases in calves (Table 1). These findings could not be compared due to lack of similar reports in the available literature.

Biochemical findings

The calcium, phosphorus, magnesium, iron, sodium, potassium and chloride were analyzed at pre-fever, fever, convalescent and recovered stages of the *E. coli* infection and the results are presented in Table 1.

Table 1. Rectal temperature and haemato-biochemical changes in calves affected with enteric and septicaemic *E. coli* infection

S/N	Parameters	Unit	Pre-fever	Fever stage	Convalescent stage		Recovered stage
			0 day	1 - 3 days	4th day	7th day	14th day	(n = 4)
			(n = 6)	(n = 6)	(n = 6)	(n = 6)	(n = 6)	
1.	Rectal temperature	^{0}F	100 – 101	103.5 – 106	101.5 – 102	100.5 – 101	100.5 - 101
			100.6 ± 0.34	**104.7 ± 0.85	101.8 ± 0.23	100.9 ± 0.19	100.9 ± 0.22
2.	Haemoglobin	g%	9.5 – 11.5	5.0 – 6.0	6.0 – 7.0	6.5 – 7.5	10.0 – 12.0
			10.50 ± 0.71	**5.58 ± 0.45	6.33 ± 0.37	7.08 ± 0.71	10.62 ± 0.82
3.	Packed cell volume	%	28 – 32	18 – 23	19 – 23	23 – 28	28 – 32
			30.5 ± 1.61	**19.83 ± 1.95	21.00 ± 1.73	25.83 ± 1.86	29.75 ± 1.48
4.	Total erythocytic count	10^6 / mm^3	5.71 – 6.94	3.11 – 4.02	3.41 – 4.52	4.59 – 6.15	5.78 – 6.24
			6.19 ± 0.38	**3.52 ± 0.58	3.98 ± 0.48	5.20 ± 0.47	6.01 ± 0.17
5.	Total leukocytic count	10^3 / mm^3	8.35 – 11.80	6.02 – 7.22	7.61 – 12.04	7.88 – 9.43	9.43 – 11.40
			9.70 ± 1.11	**6.68 ± 0.44	9.98 ± 1.62	8.74 ± 0.50	10.41 ± 0.75
6.	Serum calcium	mg / dl	8.33 – 9.61	7.98 – 8.95	8.07 - 8.67	8.19 – 8.67	8.83 – 9.67
			8.93 ± 0.42	8.43 ± 0.32	8.29 ± 0.20	8.43 ± 0.61	9.04 ± 0.36
7.	Serum phosphorus	mg / dl	6.09 – 8.13	5.74 – 8.15	3.52 – 6.11	6.19 – 7.20	7.38 – 7.93
			7.09 ± 0.68	7.34 ± 0.82	**4.99 ± 0.91	6.55 ± 0.35	7.72 ± 0.21
8.	Serum magnesium	mg / dl	2.32 – 2.38	2.37 – 2.61	2.44 – 2.60	2.41 – 2.61	2.40 – 2.47
			2.36 ± 0.02	*2.47 ± 0.08	2.52 ± 0.07	2.50 ± 0.07	2.44 ± 0.03
9.	Serum iron	µg / dl	100.32 - 111.36	107.47 - 121.09	98.70 - 129.22	102.30 - 116.88	100.32 - 115.91
			104.29 ± 3.46	*113.72 ± 5.33	107.57 ± 10.72	107.37 ± 4.94	111.04 ± 6.39
10.	Serum sodium	mmol / l	124.70 – 140.55	124.09 – 135.37	124.45 – 135.21	126.40 – 136.91	125.92 – 141.34
			135.87 ± 5.28	132.12 ± 3.87	131.12 ± 3.35	133.49 ± 3.30	134.78 ± 5.57
11.	Serum potassium	mmol / l	3.16 – 5.08	3.13 – 4.27	3.68 – 5.53	3.80 – 5.19	3.52 – 4.44
			4.05 ± 0.56	3.89 ± 0.37	4.59 ± 0.54	4.54 ± 0.55	4.02 ± 0.34
12.	Serum chloride	mmol / l	102.24 - 1.4.27[a]	101.32 - 103.72[b]	100.67 - 105.61[c]	99.09 - 104.7[d]	102.53 - 104.70[e]
			103.51 ± 0.91	102.68 ± 1.02	103.80 ± 1.66	102.70 ± 2.22	103.81 ± 8.00

[a](n = 3), [b](n = 4) [c] (n = 5) [d](n = 4) [e](n = 4), n = No. of calves, Significantly *($p < 0.05$) & **($p < 0.01$) differed from the pre-fever values.

The serum calcium concentration was insignificantly ($p > 0.05$) decreased at the peak fever stage (8.43 ± 0.32 mg / dl) in *E. coli* infected calves compared to prefever stage (8.93 ± 0.42 mg / dl) but its concentration steady increased at convalescent stage (8.29 ± 0.20 mg/dl & 8.43 ± 0.61mg / dl) and recovered (9.04 ± 0.36 mg / dl) stage (Table 1).

The serum phosphorus concentration was found significantly ($p < 0.01$) decreased at the first convalescent stage (4.99 ± 0.91 mg / dl) in calves affected with *E. coli* compared to pre-fever (7.09 ± 0.68) and fever (7.34 ± 0.82 mg / dl) stages but its steady increase was observed at the end of convalescent stage (6.55 ± 0.35 mg / dl) and become normal at the recovered (7.72 ± 0.21 mg / dl) stage (Table 1).

The serum magnesium (2.47 ± 0.08 mg / dl) and iron (113.72 ± 5.33 µg / dl) concentrations of *E. coli* affected calves showed significantly ($p < 0.05$) increased at fever stage in comparison to magnesium (2.36 ± 0.02 mg / dl) and iron (104.29 ± 3.46 µg / dl) values of the prefever stages (Table 1).

No significant ($p > 0.05$) change was observed with the serum sodium, potassium and chloride concentrations at the fever stage with that of pre-fever stage (Table 1). The different blood biochemical values determined at the prefever stage of normal calves in this study support the values of earlier reports of Pandey and Sharma (1990) who reported serum calcium 10.01 ± 0.35 mg / dl, phosphorus 5.84 ± 0.45 mg / dl and magnesium 1.73 ± 0.16 mg / dl, Kumar et al. (2000) reported calcium 10.0 ± 0.07 mg / dl, phosphorus 5.26 ± 0.12 mg / dl and magnesium 1.9 ± 0.05 mg / dl, and

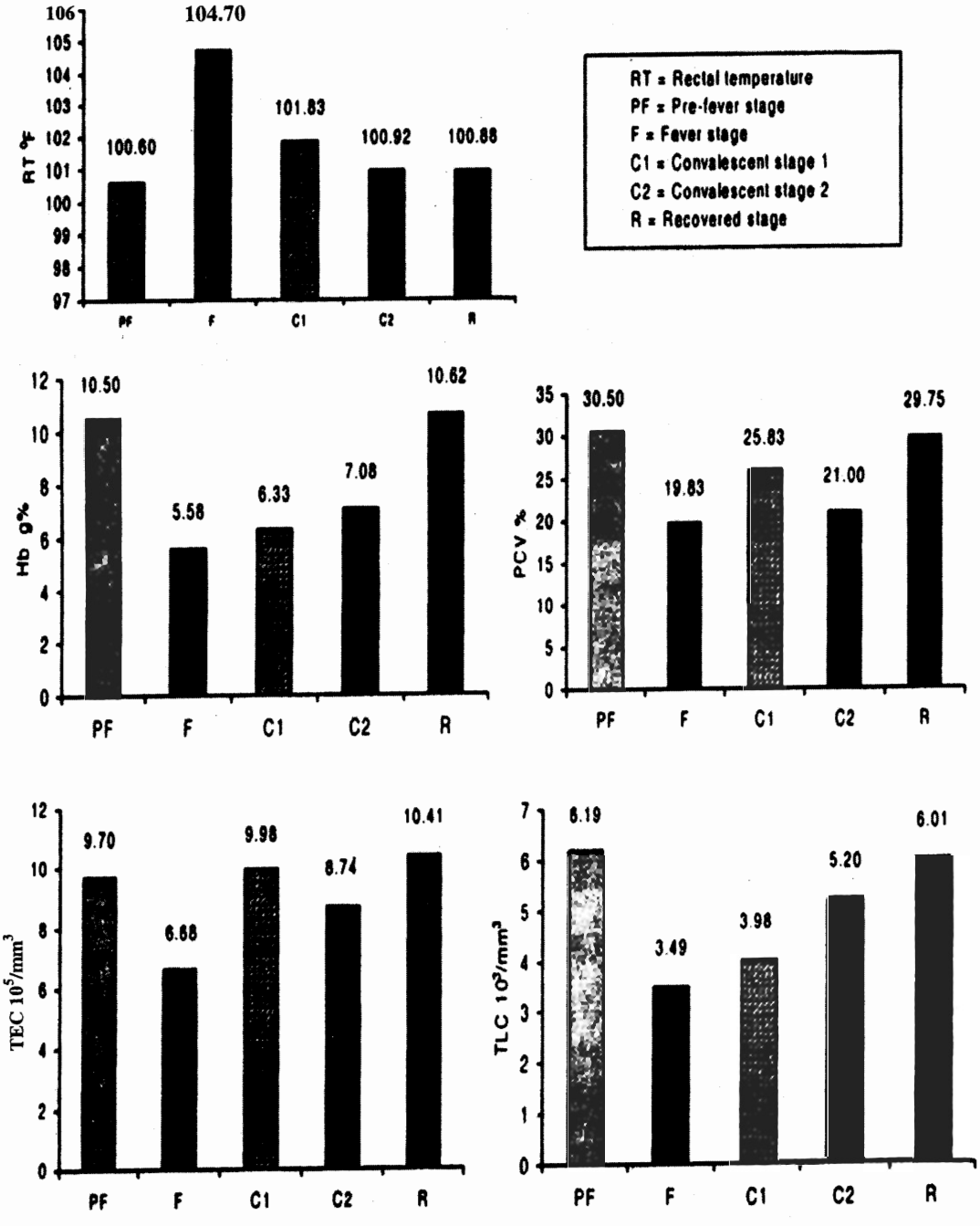

Fig. 1. Rectal temperature and haematological changes in calves affected with concurrent enteric and septicaemic *Escherichia coli* infection.

Ok *et al.* (2000) reported calcium 7.07 ± 0.7 mg / dl, phosphorus 6.59 ± 1.55 mg / dl, and magnesium 2.77 ± 0.95 mg / dl in cattle. The serum biochemical changes recorded at the different stages of *E. coli* infected calves remained uncompared due to lack of similar reports in the available literatures.

Antibiotic sensitivity

The antibiotic sensitivity pattern of *E. coli* isolated from faecal samples and blood of calves is presented in Table 2. Of the 8 isolates (6 from faeces and 2 from blood) of *E. coli* tested for sensitivity to different antibiotics showed maximum sensitivity to tetracycline, followed by streptomycin and gentamicin but it was found fully resistant to penicillin, ampicillin and amoxycilin (Table 2). The results are in conformity with the earlier report of Taleb *et al.* (2001) who reported the sensitivity to isolated *E. coli* from pneumoenteritis in calves.

Table 2. Isolation of *Escherichia coli* from febrile calves and its sensitivity to different antibiotics

Calf No.	*E. coli* isolated		Antibiotic sensitivity					
	Faeces	Blood	Tetracycline (10 iu)	Penicillin (10 µg)	Streptomycin (30 iu)	Ampicillin (6 µg)	Amoxycillin (25 µg)	Gentamicin (10 iu)
①	+	-	3+	R	2+	R	R	1+
②	+	+	3+	R	2+	R	R	1+
③	+	-	2+	R	1+	R	R	1+
④	+	+	3+	R	2+	R	R	1+
⑤	+	-	2+	R	R	R	R	1+
⑥	+	-	3+	R	1+	R	R	1+

3 + = Highly sensitive 2+ = Moderately sensitive 1+ = Less sensitive R = Resistant - = Negative

Therapeutic studies

Of the six calves affected with septicaemic *E. coli* were divided into two equal groups (Gr. A & Gr. B), each consisting of three calves. Each calf of group A was treated with Gentamicin 5%® (Techno Drugs) @ 1.0 ml / 10 kg bwt daily for 5 days, whereas each calf of group B was treated with Renamycin LA® (Renata Ltd.) @ 1.0 ml / 10 kg bwt IM with two injections at 48 hours intervals successfully.

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