HAEMATOLOGICAL STUDIES IN INDUCED BUFFALO NEONATAL CALF DIARRHOEA WITH ENTEROPATHOGENIC *E. COLI*

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ABSTRACT

This study was carried out on 24 Nili-Ravi buffalo neonatal calves divided into four equal groups and maintained at Livestock Production Research Institute, Bahadurnagar, Okara. Diarrhoea was induced with oral administration of enteropathogenic *E. coli* in first three groups while the fourth group was kept as uninfected untreated control. Twenty four hours post-infection 1st group was treated with rehydration solution and kanamycin, 2nd group with kanamycin and the 3rd group with rehydration solution. Treatment continued till the recovery or maximum up to six days. Results obtained on clinical parameters indicated green to yellow/white colour of diarrhoeic contents while consistency varied from watery to semisolid. Generally in treatment groups in about first two days, calves passed faeces around 6-10 times a day. This frequency became low in about further 2-4 days. During first 3-4 days of treatment all calves appeared weak, dull depressed, emaciated and dehydrated. Total erythrocyte counts, hemoglobin concentration packed cell volume was low in calves treated with rehydration solution + kanamycin as compared to other treatment and control group. Total leukocyte counts were high in calves treated with rehydration solution than other groups. Neutrophil and lymphocyte counts varied significantly ($P<0.05$) among all treatment and control group. Moderate to severe emaciation and dehydration was present in calves treated with only antibiotic but these changes were less prominent in calved treated with rehydration solution alone or in combination with antibiotic.

Keywords: Buffalo neonatal calf diarrhoea, experimental trials, enteropathogenic *E. coli*, haematological studies

INTRODUCTION

For a profitable livestock industry successful calf rearing is the fundamental requirement. Unfortunately a large number of calves die before reaching their first birthday causing heavy drain to the economics of livestock industry. Mortality in buffalo and cow neonatal calves in Pakistan has been reported to be 11.0 and 7.1 per cent, respectively. Mortality due to diarrhoea varies from 23.7 to 63.0 per cent in buffalo and 26.0 to 36.84 per cent in cows neonatal calves (Khan and Khan, 1996a).

Mortality among neonatal calves is not only the problem but also leads to over 30 per cent sharp decline in buffalo milk production (McDowell *et al.*, 1995). Diarrhoea in infancy results in future reduction of meat volume (20%) and dairy production (18%). Delayed growth and development and reproductive dysfunctions are observed in those cattle having diarrhoea in infancy (Pilui, 1984). The percentage of infertility is twice high in those cattle having diarrhoea in infancy. In addition the general non-specific resistance of animal is reduced facilitating the incidence of other diseases (Shakis, 1985). According to an estimate, 20 per cent calf mortality can reduce net profit upto 38 per cent (Blood and Radostits, 1989).

Etioloty of neonatal calf diarrhoea is complex, it may be infectious or non-infectious in origin. The infectious agents causing diarrhoea include, enteropathogenic *E. coli*, rotavirus, salmonella species, coronavirus and cryptosporidium (Wernicki and Rzedzicki, 1988). Non-infectious etiology could be due to bad management, environmental factors, parity of dam etc. Enteropathogenic *E. coli* has the highest incidence (55.6%) than salmonella spp (13.3%) (Khan and Khan, 1997a). The possible phenomenon of diarrhoea development in neonatal calves seems to be caused by enteropathogenic *E. coli*, adhering to mucosa and proliferating in the lumen of intestine, there by producing a potent enterotoxin, which stimulate excessive secretion of fluid from intestinal mucosa causes the principal sign diarrhoea which often leads to imbalance of electrolytes, resulting in high rates of death. It has been reported that a loss of as much as 15 per cent of body fluid results in death (Khan and Khan, 1997b).

In buffalo neonates, references are available on incidence (Afzal *et al.*, 1983; Khan and Khan, 1996a) and aetiopathology (Hafiz *et al.*, 1994; Khan and Khan, 1997 a) of neonatal calf mortality. Much work has been carried out on treatment of cow neonatal calf diarrhoea in western countries, however, such information in buffalo neonates is sparse, therefore, the present project was carried out to know i) the effect of induced diarrhoea by enteropathgenic *E. coli* on haematological changes and ii) to compare different treatment regimens to find out the most effective treatment.

MATERIALS AND METHODS

Animals and management

Twenty four about a week old buffalo neonates were procured from local market and maintained at LPRI (Livestock Production and Research Institute) Bahadurnagar. These calves were kept for 4 days in a group to acclimatize and whole milk @ 10 per cent body weight was fed. Faecal samples from each neonate was collected to check for the presence of any pathogen, on day 4th neonates free of infection were divided into four equal groups.

Induction of infection, treatment protocol and composition of rehydration solution has been described else where (Javed *et al.*, 2000). First group(G-1) was treated with rehydration solution plus kanamycin, 2nd group (G-2) with kanamycin and the 3rd group (G-3) with rehydration solution till the recovery or maximum up to six days. Fourth group (G-4) served as control.

Sample collection and analysis

Blood samples were collected at 12 hours interval for three days post-infection then at interval of 24 hours for three days. These samples were studied for various haematological parameters such as total erythrocyte counts (TEC), haemoglobin concentration (Hb. Conc.), packed cell volume (PCV), erythrocyte sedimentation rate (ESR), total leukocyte counts (TLC) and differential leukocyte counts (DLC) following the method described by Benjamin (1979). Blood pH was also determined with the help of pH meter.

Data analysis

Data thus collected was subjected to analysis of variance and means were compared by using LSD test on personal computer by using SAS 6.12 software package (Anonymous, 1996).

RESULTS

Diarrhoea developed in all treatment groups (1-3) in 2 to 6.30 hours after oral infection of enteropathogenic *E. coli*. The nature of diarrhoea contents varied from semisolid to watery. The colour of diarrhoeic fluid was yellowish to greenish mixed with blood flakes or mucous. The frequency of defecation also varied from 6-10 times per day.

Recovery pattern

In group 2 (Kanamycin) two calves died in spite of treatment. In other groups no mortality was observed. Recovery in group I (rehydration solution + kanamycin) started from the third day, three calves were fully recovered from dehydration. All physical parameters were restored. In group 2 (Kanamycin), recovery was some what and started from 5th day. Calves treated with rehydration solution (group 3), recovery pattern was also quicker as compared to calves treated with kanamycin.

Haematological findings

Mean TEC, Hb. conc. and PCV revealed significant ($P<0.05$) difference between treatment and control group. Values of these parameters were significantly ($P<0.05$) higher in G-4 calves than G-1 (Table 1). Mean ESR revealed non-significant difference between treatment and control group. Rouleaux formation is a normal feature of buffalo calves. Diarrhoeic neonates treated with kanamycin showed numerous crescent shaped cells (Plate 1), anisocytosis (Plate 2) and crenation (Plate 3).

Mean TLC counts of G-1 and G-2 varied significantly ($P<0.05$) from G-3 and G-4 calves. However, non-significant difference was observed in G-3 and G-4 and in G-1 and G-2 calves (Table 1).

Mean neutrophilic and lymphocytic counts in treatment groups (1-3) varied significantly ($P<0.05$) from control group. Neutrophils were significantly ($P<0.05$) higher in G-4 and significantly ($P<0.05$) lowered in G-1 (Table 1). Lymphocytes were significantly ($P<0.05$) higher in all treatment groups (1-3) as compared to control group (Table 1). Mean monocyte, eosinophil and basophil counts varied non-significantly in all four groups (Table 1).

Blood pH varied significantly ($P<0.05$) between treatment and control group and variation in treatment groups (1-3) were non-significant.

Data analysis revealed that various parameters in different groups have correlation. Group treated with rehydration solution + Kanamycin (G-1) showed positive correlation between Hb. Conc. and TLC ($r=0.382$, $P < 0.031$) whereas negative correlation between ESR and TEC ($r=-0.424$, $P<0.016$), neutrophils and lymphocytes ($r=-0.992$, $P< 0.031$). Group treated with Kanamycin (G-2) showed positive correlation between TLC and neutrophils ($r=0.403$, $P<0.051$). Lymphocytes showed negative correlation with neutrophils ($r=-0.996$, $P<0.0001$). The pH showed positive correlation with ESR ($r=0.472$, $P< 0.020$) and Hb. Conc. ($r=0.548$, $P<0.006$). In diarrhoeic calves (G-3) treated with rehydration solution, ESR showed positive correlation with neutrophils ($r=0.483$, $P<0.004$) and negative correlation with lymphocytes ($r=-0.496$, $P< 0.04$).

Table 1: Haematological parameters in control and treatment groups of diarrhoeic buffalo neonatal calves.

Parameters	G-1	G-2	G-3	G-4
Total erythrocyte count (x $10^6/\mu L$)	7.23 ± 1.71 B	8.13 ± 1.98 AB	8.65 ± 1.84 AB	8.83 ± 2.60 A
Haemoglobin concentration (g/dL)	11.83 ± 2.24 B	12.65 ± 3.08 AB	13.11 ± 1.97 AB	14.08 ± 2.98 A
Packed cell volume (%)	33.75 ± 6.07 B	36.44 ± 5.18 AB	36.92 ± 4.33 AB	35.52 ± 5.24 AB
Erythrocytes sedimentation rate (mm/24 hours)	65.88 ± 26.30	55.46 ± 28.51	67.72 ± 24.87	65.76 ± 23.00
Total leukocyte count (x $10^3\mu L$)	10.37 ± 2.57 AB	9.31 ± 3.1 B	12.09 ± 3.45 A	11.96 ± 3.14 A
Neutrophils (%)	54.58 ± 5.84 A	53.41 ± 4.82 A	55.35 ± 6.80 A	44.71 ± 3.04 B
Lymphocytes (%)	40.86 ± 3.12 A	41.09 ± 2.24 A	40.52 ± 3.78 A	50.94 ± 6.56 B
Monocytes (%)	3.05 ± 2.02	3.61 ± 1.88	2.75 ± 2.13	3.00 ± 2.24
Eosinophils (%)	1.51 ± 1.01	1.89 ± 1.21	1.38 ± 1.00	1.35 ± 0.71
Basophils (%)	0.31 ± 0.47	0.17 ± 0.36	0.09 ± 0.28	0.10 ± 0.28
Blood pH	6.78 ± 0.19 B	6.75 ± 0.22 B	6.78 ± 0.18 B	6.90 ± 0.18 A

Values bearing different letters in a row differ significantly (<0.05);
G-1 = rehydration solution + Kanamycin; G-2 = Kanamycin; G-3 = rehydration solution; G-4 Uninfected untreated control

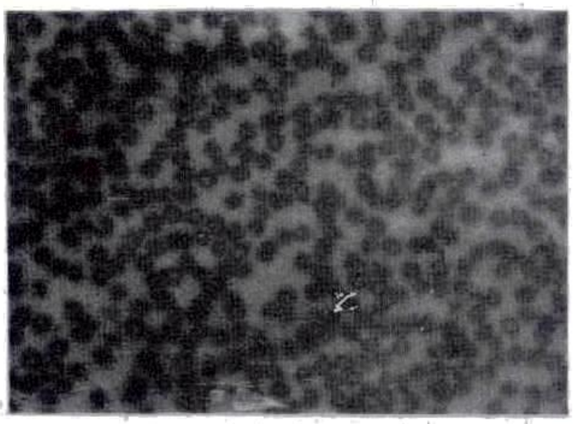

Plate 1: Photomicrograph showing numerous crescent shape cells in blood smear prepared from diarrhoeic buffalo neonatal calf (Giemsa's, 200 x).

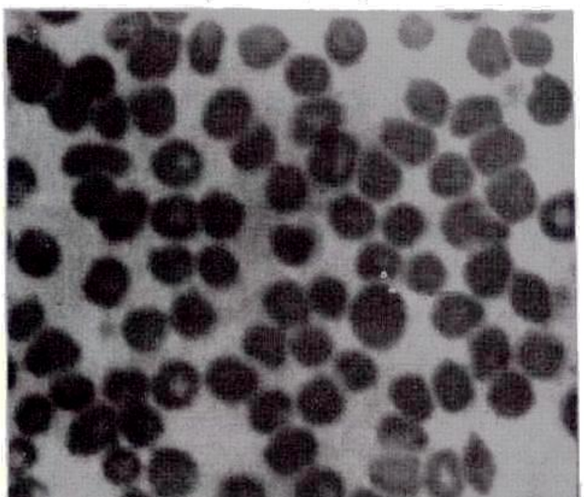

Plate 2: Photomicrograph showing anisocytosis in blood smear prepared from diarrhoeic buffalo neonatal calf (Giemsa's, 1000 x).

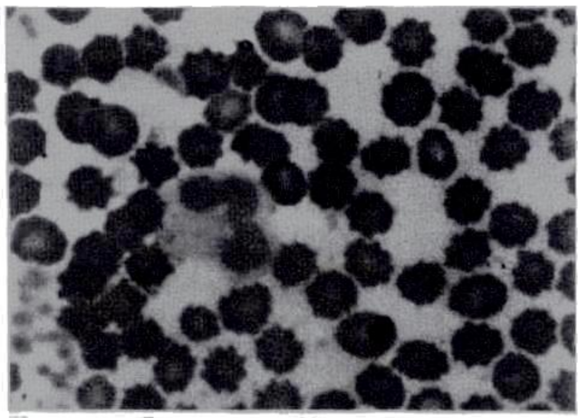

Plate 3: Photomicrograph showing extensive crenation and bowl cells in blood smear prepared from diarrhoeic buffalo neonatal calf (Giemsa's, 1000 x).

DISCUSSION

Neonatal calf mortality is mainly attributed to neonatal calf diarrhoea. The etiology of neonatal calf diarrhoea is cimplex. Many infectious agents (Snodgrass *et al.*, 1986) and non-infectious factors contribute to the disease process (Roy, 1980). The role of enteropathogenic *E. coli* in neonatal buffalo calf diarrhoea has been investigated (Hafiz *et al.*, 19994; Khan and Khan, 1997a). Enteropathogenic *E. coli* adheres to the mucos, proliferates in the intestinal lumen and produce a potent enterotoxin stimulating excessive fluid secretion (Khan, 1994). The secretion of this fluid causes diarrhoea which results in blood acid base imbalance, electrolyte depletion and hypovolaemia consequently resulting into shock and death (Rousal and Kasari, 1990). According to Khan and Khan (1997b) as much as 15 per cent loss of fluid solution results in death. Timely treatment with oral rehydration solution (ORS) and antibiotics alone or in combination with ORS proved successful in reducing the calf mortality (Holck *et al.*, 1994; Brooks *et al.*, 1996). The key element of therapy in severely diarrhoeic calves is the rapid intravenous restoration of salt and water depletion (Kaske, 1994).

During present study the local enteropathogenic *E. coli* strain was isolated from field cases of diarrhoea and was administered (1010 CFU per 2 ml) orally to induce diarrhoea in buffalo calves. The diarrhoea started within 4-6 hours of experimental infection to all calves in group 1-3. Calves treated with antibiotic alone (G-2) showed severe hemoconcentration as compared to other treatment groups (G-1 and G-3). Diarrhoea culminates into dehydration which leads to hemoconcentration (Al-Ani, 1992; Maach *et al.*, 1992). Increased TEC, Hb.Conc. and PCV were observed in calves treated with rehydration solution as compared to other groups (G-1 and G-2). As frequency of diarrhoea was more in former group, therefore, hemoconcentration developed which leads to higher TEC, Hb.Conc. and PCV. Similar observations have been reported by Mert *et al.* (1990), Desphande *et al.* (1993), Aly *et al.* (1996) and Khan and Khan (1996b).

Decrease in TEC, Hb. conc. and PCV were oberved in low dehydrated diarrhoeic calves has been reported by Mert *et al.* (1990). This is why low TEC, Hb. Conc. and PCV were observed in G-1 calves which were treated with rehydration solution + antibiotic in the present study, as these calves were low dehydrated. The treatment of rehydration solution + antibiotic was started well in time, therefore, severe dehydration did not develop.

Total leukocyte counts (TLC) was significantly ($P<0.05$) higher in calves treated with rehydration solution as compared to other groups (G-1 and G-2) in the present study. In response to infectious agents in the body, leukocytosis takes place, similarly in the present study enteropathogenic *E. coli* was introduced orally for induction of diarrhoea. Salania *et al.* (1985), Mert *et al.* (1990) and Aly *et al.* (1996) also reported leukocytosis in the diarrhoeic calves.

Leukocytosis was mainly due to neutrophilia in the G-3 calves whereas lymphocytopenia was also present. Hoerstke *et al.* (1982) and Sridhar *et al.* (1980) reported neutrophilia and Lymphocytopenia in diarrhoeic calves. Other leukocytic series cells did not show any response to the infection. Blood pH decreased (acidosis) in all treatment groups due to diarrhoea as compared to control group. Similar observations have been reported by Michell *et al.* (1992), Michna *et al.* (1996) and Brooks *et al.* (1996).

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