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Haemato- biochemical changes associated with neonatal calf diarrhoea caused by EPEC

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Abstract
Present study was done for the detection of enetropathogenic E. *coli* (EPEC) induced diarrhoea in neonatal calves and analysis of haemato-biochemical changes during the disease. Genes for production of the virulence factor Intimin was detected by PCR in DNA extracts of faecal samples from six out of 50 diarrhoeic calves. Haematology of affected calves revealed anaemia (VPRC 20.27 per cent) and pan leukocytosis (mean TLC 13533cells/mm^3). Biochemical analysis of serum showed hyperlacticaemia (2.606 mmol/L) and hyponatraemia (137.167 mmol/L). Hypoalbuminaemia (1.415 g/dl) and decrease in level of A: G ratios (0.539) were the two other prominent variations. No other significant alterations could be detected in any other clinical or haemato-biochemical parameters.

Keywords: Enteropathpogenic E. coli, Haemato-biochemical changes, neonatal calves

1. Introduction
Neonatal calf diarrhoea (NCD) has a worldwide occurrence with multifactorial etiological agents including bacteria, virus and protozoa [1]. Among the *E. coli*, enter toxigenic *E. coli* (ETEC) is considered as the major one [2]. While enetropathogenic *E. coli* (EPEC) is considered as an emergent pathogen [3]. In case of EPEC, the gene known as *eaeA* produces an outer membrane protein called intimin which causes the attaching and effacing lesions in the host leading to the disease [4]. This organism also possesses zoonotic potential and calves can act as a source of infection.
Neonatal calf diarrhoea is associated with haemato-biochemical alterations often leading to the death of the affected calf. These changes noticed in diarrhoeic calves are complex in nature often resulting in serious imbalances of fluid, electrolyte and acid base status, and thereby threatening the life of the patient.

2. Materials and methods
2.1 Selection of animals
Fifty neonatal calves (less than 30 days of age) with diarrhoea from different government and private farms in and around Thrissur district, Kerala, India were the subjects of this study.

2.2 Collection of samples
Faecal samples directly from the rectum and 10 ml of blood from the jugular vein were collected from each calf. These samples were transported to the laboratory under refrigeration.

2.3 Processing of sample
Faecal samples were subjected to DNA extraction using commercially available DNA extraction kits (QI Aamp® DNA Stool Mini Kit, QIAGEN, Germany). Heparinized blood samples were subjected to blood gas analysis (Epoc™ Blood Gas analyzer, Alere, Australia). Blood samples collected in EDTA vials were used for haematological analysis using automatic blood analyser (Orphee, Mythic Vet 18, UK) and the serum samples were subjected to bio-chemical analysis using semi-automatic biochemical analyser (Erba Manheim, Chem-5 Plus v2, USA).

2.4 Detection of E. coli
Extracted DNA from each sample was subjected to pathogen specific PCR for *eaeA* gene [2]. Product was viewed after electrophoresis and imaged using Gel doc for observing amplified products with corresponding product size. The products were further subjected to sequencing

And homology with documented genotypes was confirmed by using BLAST software of NCBI.

3. Results and discussion

EPEC was an emerging cause of neonatal calf diarrhoea [3]. The affections cause serious haemato-biochemical changes and dehydration due to the virulence factors. Bacteria get attached on the surface of intestinal epithelium with the formation of attaching and effacing lesions causing reduction in the absorptive capacity of the intestinal mucosa, which leads to disruption of the electrolyte balance and subsequently resulting in diarrhoea [5].

Out of 50 neonatal diarrhoeic calves six (12 per cent) were found to possess the *eaeA* gene for intimin, indicating presence of EPEC [2]. The animals were showing mild to moderate dehydration. All animals were having watery mucoid diarrhoea with one calf exhibiting greenish black coloured stool.

The haemato- biochemical variations observed in calves with NCD caused by EPEC are listed in the table- 1. Infection by foreign pathogenic agent causes an appropriate defence response in body. These changes were reported earlier [6, 7]. Leukocytosis was the important haematological change observed in this study. Total erythrocyte count, VPRC and haemoglobin levels are reported to be higher [7], but not in the present study.

Hyperproteinemia associated with dehydration, inflammatory hypergamaglobulinemia and relative hypoalbuminemia were the three major changes reported in NCD [8]. Hyponatremia was the major variations from normal observed in the present study. There was also an increase in blood lactate level, similar to reports in claves with to metabolic acidosis [9]. Significant hypoalbuminemia and decrease in level of A: G ratio was evident in this study. An increased level of serum creatinine was reported in NCD [8], which was evident in our study also.

Table 1: Haemato- biochemical changes of EPEC infected diarrhoeic animals

Haemato- biochemical parameters	Mean value	Reference interval
Na+ 3 (mM)	137.1667	145-160
K+ (mM)	4.1	4.8-5.8
Cl− (mM)	101.8333	92-104
HCO3(mM)	25.01667	28.0–36.9
Glucose (mg/dl)	92.83333	74- 100
Lactate (mmol/L)	2.606667	0.56- 1.39
Total leucocyte count (x10^3/ mm^3)	13.53333	4.7–11.4
Lymphocyte (x 10^3/ mm^3)	7.716667	1.9–5.9
Monocyte (x 10^3/ mm^3)	0.816667	0.2–0.8
Granulocyte (x 10^3/ mm^3)	5.016667	1.3–5.3
Total Erythrocyte count (x 10^6/ mm^3)	6.12	4.6–6.9
Haemoglobin (g/dl)	6.666667	8.8–12.5
VPRC (per cent)	20.7	40–53
Total protein (g/dl)	4.21	4.70–6.87
Serum Albumin (g/dl)	1.415	2.68–3.66
Serum Globulin (g/dl	2.795	1.61–3.61
Albumin: Globulin	0.539563	0.78–1.74
Creatinine (mg/ dl)	1.3	0.59–1.28

Conclusion

Present study conclude the major haemato- biochemical changes in EPEC infected neonatal diarrhoeic calves were leucocytosis, hypoalbuminemia, hyperglobulinemia, hyperlactimia, decreased haematocrit and A: G ratio.

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