

Role of Metabolic Acidosis and Alteration in Blood Gas and Electrolyte Levels in Colibacillosis Affected Calves of Wayanad District, Kerala

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Abstract

Colibacillosis is an economically important disease, causing neonatal diarrhoea and septicaemia in calves. The present study was carried out in the Department of Veterinary Epidemiology and Preventive Medicine. A total of thirty neonatal calves affected with diarrhoea were selected from Wayanad district. The present study was conducted to identify colibacillosis in calves from Wayanad district by identification of clinical signs such as diarrhoea, pyrexia, severe dehydration, etc., Faecal sample culture in MacConkey' s agar and Faecal culture in EMB agar. To identify the level of metabolic acidosis bicarbonate level of venous blood was analyzed using Epoc® Blood Gas Electrolyte Analyzer. Significant increase in pCO₂, lactate and creatinine were observed. significant reduction in pH, pO₂, base excess (BE), Na⁺, K⁺, Glucose, Bicarbonate and chloride were significantly lower in test group comparing with control group (p ≤ 0.01). There was no significant difference between test group and control group in Ca⁺ levels.

Keywords: Bacterial Culture, Blood Gas Electrolyte Analysis, Colibacillosis, Neonatal Calf Diarrhoea, Metabolic Acidosis, Statistical Analysis Based on Independent Sample T- Test



Introduction

Colibacillosis is an economically important disease in livestock especially neonatal calves. More than 50 per cent mortality is observed in calves because of this condition. Six important categories of pathogenic *E. coli* which are, enteropathogenic *E. coli*, enterohaemorrhagic *E. coli*, enterotoxigenic *E. coli*, enteroaggregative *E. coli*, enteroinvasive *E. coli* and diffusely adherent *E. coli* are responsible for colibacillosis in animals. Out of them enterohemorrhagic *E. coli*, enteropathogenic *E. coli*, and enterotoxigenic *E. coli* cause colibacillosis in calves. As *E. coli* bacteria is an environmental pathogen, almost all farms are in the risk of this disease. Common clinical signs associated with colibacillosis are diarrhoea, dehydration, metabolic acidosis and pyrexia. Sometimes complication like meningitis and arthritis could occur. Mortality is associated with level of dehydration and metabolic acidosis. So, identification of level of metabolic acidosis and level of dehydration serves a great role in diagnosis and treatment of colibacillosis. Changes in clinical and blood gas and electrolytes associated with colibacillosis in neonatal calves of Wayanad district in Kerala are targeted in current research work.

Holt-Harris and Teague (1916) developed a new culture medium based on methylene blue and eosin for *Bacillus typhosus*. This media was known as Eosin Methylene Blue (EMB), was effective for the isolation of *E. coli*. Frequent and effortless defecation, hind quarter usually pasted with fluid and semisolid faeces were usually associated with enteric colibacillosis. Faeces would be malodorous, fluidly, containing partially digested milk, yellow-white or grey in colour. Rectal temperature was normal in initial stages but getting subnormal in advanced conditions (Haggard, 1985). Metabolic acidosis in neonatal calves with and without diarrhoea was because of hyponatremia accompanied by normochloremia or hyperchloremia, D-lactate, and an increase in plasma protein concentration. Ideal fluid for fluid therapy should contain a high sodium concentration and a high effective strong ion difference. (Constable *et al.*, 2001). One per cent glucose added to violet red bile agar is termed as MacConkey's agar. This agar can be used for the identification of coliform bacteria (Fleming *et al.* 2001). Lorenz (2004) conducted a study on the relationship between D-lactate and metabolic acidosis in diarrheic neonatal calves and found out that increased D-lactate concentration could provide clinical picture of metabolic acidosis. Radostits *et al.* (2007) reported that common clinical signs associated with colibacillosis were diarrhoea, dehydration, metabolic acidosis and pyrexia. Sometimes complication like meningitis and arthritis could occur. Trefz *et al.* (2011) conducted a study on metabolic acidosis in neonatal calf diarrhoea and concluded that the degree of metabolic acidosis in diarrhoeic calves could be predicted based on postural and behavioral alterations. Degree of loss of palpebral reflex could be a trusted tool to predict D-lactate concentration. Singh *et al.* (2014) discovered that there was a significant increase in packed cell volume of colibacillosis affected neonatal calves but there was no significant alteration in haemoglobin. A prevalence study on enteropathogens causing neonatal diarrhoea in calves in Thrissur district of Kerala revealed a seroprevalence of 42.1 per cent for Rotavirus, 26.3 per cent for Coronavirus, 36.8 per cent for *E. Coli* and 28.9 per cent for *Cryptosporidium parvum* (Janus *et al.*, 2015). Bednarski *et al.* (2015) reported a significant decrease in (partial pressure of CO₂) pCO₂ in calves with diarrhoea. Amrutha. *et al.* (2018) found out an incidence of 12 per cent for enteroaggregative *E. coli*, eight per cent for enterohaemorrhagic *E. Coli* and four per cent for Shiga-toxin producing *E. Coli* from diarrheic calves in Thrissur district of Kerala.

Materials and Methods

The present study was carried out in the Department of Veterinary Epidemiology and Preventive Medicine, between May 2018 and April 2019. A total of thirty neonatal calves affected with diarrhoea were selected from those presented at Teaching Veterinary Clinical Complex, CVAS, Pookode, dairy farms and rural households in Wayanad district.

Clinical Examination

Based on detailed clinical examination the vital parameters such as respiration (rate/min), body temperature (°F), pulse (rate/min), heart rate (rate/min), skin tending (sec), depth of enophthalmos (mm) and per cent of dehydration were assessed. The hydration status of animals was assessed as per the protocol of Radostits *et al.* (2007).

Blood and Faecal Sample

Blood (0.5 ml) from jugular vein of calf was taken and blood gas electrolyte analysis was done by epoc® Blood Gas Electrolyte Analysis System and results were recorded. Faecal sample were collected directly from anus and

transported to laboratory for identification of *E. coli* by culture.

Blood Gas Electrolyte Analysis

Blood gas electrolyte analysis was done using E poc® Blood Gas Electrolyte Analysis System. pH, pCO₂ (mm of Hg), pO₂ (mm of Hg), HCO₃⁻ (mmol/L), BE (mmol/L), Na⁺ (mmol/L), K⁺ (mmol/L), Cl⁻ (mmol/L), Ca⁺⁺ (mmol/L), Glucose (mg/dL), Lactate (mmol/L) and Creatinine (mg/dL) were recorded. The value of base excess was utilized in the diagnosis and treatment of metabolic acidosis. The process was done with E poc® Card and E poc® Blood Gas Electrolyte Analyzer.

Statistical Methods

Independent sample t-test was used to analyze the data (Pandey R.M, 2015)

Results and Discussion

Clinical Findings

Based on the detailed clinical examination, vital parameters and hydration status were recorded and results with statistical analysis are given in Table 1. Mean value of vital parameters viz., body temperature, pulse, heart rate, skin tend time requirement, depth of enophthalmos and per cent of dehydration indicated a marked elevation in those vital parameters. Independent sample t test revealed vital parameters viz., body temperature, pulse, heart rate, skin tend time requirement, depth of enophthalmos and per cent of dehydration were significantly different from vital parameters of control population. Respiratory rate was significantly lower than that of control group. Clinical signs including diarrhoea, bradypnoea, pyrexia, tachycardia and dehydration were noted in comparison with control group which was in accordance with Radostits *et al.* (2007). Blood mixed, mucoid, clay coloured, yellowish and watery types of diarrhoea were encountered in this study, which was almost in 73 agreement with the findings of Haggard (1985). Almost all the cases were having metabolic acidosis due to HCO₃⁻ loss and base deficit (Trefz *et al.*, 2012).

Table 1: Clinical findings from sample population

Parameter	Calves with diarrhea	Calves in control group	P value	t value
Respiration (rate/min)	21.93 ± 0.68	24.42 ± 0.36	0.007	1.721**
Body temperature(°F)	103.1 ± 0.27	101.05 ± 0.19	0.046	6.095*
Pulse(rate/min)	68.2 ± 1.02	64.28 ± 0.6	0.01	1.815**
Heart rate(rate/min)	68.2 ± 1.02	64.29 ± 0.61	0.01	1.815**
Skin tending(sec)	6.03 ± 0.44	0.69 ± 0.06	0.001	5.719**
Depth of enophthalmos(mm)	3.933 ± 0.42	0	0.001	4.385**
Per cent of dehydration	7.767 ± 0.73	0	0.001	5.085**

**= Statistically significant at 1 percent level, *=Statistically significant at 5 percent level and ns= Statistically not significant



Figure 1: Lactose fermented pink colonies in MacKonkey's agar

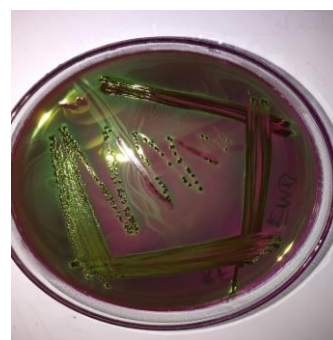


Figure 2: Purple colonies with black centre and green metallic sheen in EMB agar.

Identification of *E. Coli* by culture

All the thirty sample were positive for lactose fermented pink colonies in MacKonkey's agar (Figure 1) and purple colonies with black centre and green metallic sheen in EMB agar (Figure 2).

Blood Gas Electrolyte Analysis

There was significant increase in pCO₂, lactate and creatinine in test population in comparison with control group (p≤ 0.01) but pH, pO₂, base excess (BE), Na⁺, K⁺, glucose were significantly lower in test population than that of control group (p0.001). Bicarbonate and chloride were significantly lower in test group comparing with control group (p≤ 0.01). There was no significant difference between test group and control group in Ca⁺. Data obtained from blood gas electrolyte analysis were subjected to independent sample t test and results were recorded in Table 2.

Table 2: Results of blood gas electrolyte analysis

Parameter	Mean value with Standard Error		P-value	t- value
	Test	Control		
Ph	7.26 ± 0.05	7.43 ± 0.02	0.008	2.792**
pCO ₂ (mmHg)	49.01 ± 1.74	39.01 ± 1.34	0.011	2.7**
pO ₂ (mmHg)	81.86 ± 1.90	93.98 ± 1.79	0.001	4.63**
HCO ₃ -(mmol/L)	21.72 ± 1.11	27.21 ± 1.24	0.044	2.301*
BE(mmol/L)	-6.53 ± 4.99	5.07 ± 1.37	0.002	3.604**
Na+(mmol/L)	93.17 ± 3.55	124.43 ± 6.65	0.002	4.141**
K+(mmol/L)	7.7 ± 0.34	15.22 ± 1.35	0.014	7.924**
Cl-(mmol/L)	84.29 ± 2.92	104.23 ± 1.11	0.05	2.188*
Ca+(mmol/L)	1.49 ± 0.05	1.26 ± 0.09	0.065	2.076 ^{ns}
glucose(mg/dl)	97.77 ± 2.71	118.14 ± 5.21	0.007	3.466**
Lactate(mmol/L)	1.18 ± 0.08	0.91 ± 0.04	0.031	1.534*
Creatinine(mg/dL)	2.08 ± 0.25	1.09 ± 0.07	0.001	3.684**

**= Statistically significant at 1 percent level, *= Statistically significant at 5 percent level and ns= Statistically not significant

Blood gas electrolyte analysis revealed a significant increase in pCO₂ which is contrary to the findings of Bednarski *et al.* (2015) who reported a significant decrease in pCO₂. Radostits *et al.* (2007) reported increase in pCO₂ level in neonatal colibacillosis in calves. In the current research a marked increase in D- lactate concentration and base deficit were reported which is in accordance with Lorenz (2004). The D-lactate accumulation was caused by increased intestinal absorption of D-lactate and marked base deficit was due to increased HCO₃⁻ loss through diarrhoea.

The present study results revealed a significant elevation in creatinine level which was due to acute renal failure caused due to severe dehydration. This result was in accordance with Singh *et al.* (2014) In the present study hyponatremia was encountered which could be due to marked loss of sodium through diarrhoea. The results are in agreement with Constable *et al.* (2001). Hypokalemia observed in the present study is in agreement with Sobiech and Kuleta (2006) who reported hypokalemia in calves with diarrhoea. The present research revealed marked decrease in glucose, HCO₃⁻ and chloride levels in diarrhoeaic calves, and these conditions led to hypoglycemia in diarrhoea and anorexia. Calves were unable to drink enough milk and ingested milk always lose through diarrhoea. This leads to impaired glucose assimilation and leads to hypoglycemia. Bicarbonate and chloride levels were significantly lower in the test group compared with the control group, these results were because of excess loss of HCO₃⁻ and chloride loss through diarrhoea and the present results were well in accordance with Trefz *et al.* (2011).

Conclusion

E. coli bacteria are most common and important health hazard in both human health sector and veterinary sector. To control deaths associated with colibacillosis, it is essential to identify alterations in blood gas electrolyte parameters associated with the disease. So, current study was targeted on blood gas electrolyte abnormalities associated with colibacillosis in neonatal calves. Results were hypercapnoea, marked increase in D- lactate concentration and base deficit, hyponatremia, hypokalemia, hypoglycemia, marked decrease in HCO₃⁻ and chloride

levels. Most important finding was incidence of metabolic acidosis in all calves. These results suggest requirement of intensive fluid therapy with sodium bicarbonate, dextrose and electrolyte solutions.

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Conflict of Interests

There is no conflict of interest.

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