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Alterations in clinical and haemato-biochemical profile of diarrhoeic cattle calves due to *E. coli* and *Salmonella* spp.

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Abstract
Diarrhoea is an important disease of economic importance because of high morbidity and mortality and major cause of deaths from infectious diseases in cattle calves. Due to its adverse effects on the herd's longevity, production, and immediate health, it has an adverse economic impact on livestock farmers. The present study was conducted to access the alterations of clinico-haemato-biochemical parameters in diarrhoea affected cattle calves attributed to *E. coli* and *Salmonella* spp. infection. Forty cattle calves below 3 months of age irrespective of sex and breed were selected, 10 animals apparently healthy as control group, the affected 30 animals with apparent clinical signs of diarrhoea were considered as infected group. Clinical parameters were recorded and blood as well as sera samples were collected from both groups. Clinical scores (0-3 basis) revealed healthy calves were scored as 0, whereas among diarrhoeic calves, 8 (26.67%) were with clinical score of 1, 17 (56.67%) were with clinical score of 2 and 5 (16.66%) were with clinical score of 3 and not significantly different heart rate, respiration rate, or rectal temperature. Hematology revealed significant higher values Hb, PCV, TLC, TEC and neutrophil (%), while significant lower value of Lymphocyte (%) in diarrheic calves. Serum biochemical analysis revealed significant higher values of total protein, albumin, globulin, A:G ratio and potassium while significant lower values of sodium, chloride, bicarbonate and glucose in diarrheic calves. The results of this study indicated that *E. coli* and *Salmonella* spp. causes diarrhea in cattle calves which in turns alters the clinical, hematological and biochemical profile. This study helps to understand the Pathophysiology of diarrhoea due to *E. coli* and *Salmonella* spp. and helps in adopting a good management and treatment strategy in diarrhoeic cattle calves.

Keywords: *E. coli, Salmonella* spp., calves, clinico-haemato-biochemical parameters

1. Introduction
One of the most prevalent intestinal infections in young animals is calf diarrhea, which costs the global dairy sector enormous sums of money in lost productivity and progeny (Cho and Yoon, 2014) [4]. Diarrhea in calves is most common during the first week of life and is associated with electrolyte and water loss, which can cause hypotonic or isotonic dehydration (Azizzadeh *et al*., 2012) [1]. The rectal temperature, pulse, and respiration rate were all within the normal range (Ramkumar, 2012) [16]. The majority of enteropathogens, including *Salmonella*, Clostridium, and E. coli species, have been corresponding to calf diarrhea (Singla *et al*., 2013; Cho, and Yoon, 2014) [20, 4]. The complicated clinico-haematobiochemical changes associated with diarrhea include severe fluid, electrolyte, and acid-base abnormalities that endangered the survival of the affected calves. To identify possible risk factors and infection sources, it is crucial to identify the probable causal agent in diarrhea outbreaks (Izzo *et al*., 2011) [10]. Our study was conducted to determine alterations in the clinical and hemo-biochemical profile in cattle calves below 3 months of age. *Salmonella* spp. and E. coli are the most common causes of diarrhea in dairy calves.

2. Materials and Methods
Cattle calves below 3 month of age irrespective of sex and breed showing classical clinical signs of diarrhoea at brought to Medicine Clinic of Veterinary Clinical Complex, of

RAJUVAS Bikaner were source of animals for the present study. Faecal examination were conducted using direct smear, sedimentation and Willi's floatation techniques, to exclude the gastrointestinal parasites infested calves from this study on the basis of presence of parasite or parasitic segments, eggs, cysts and oocysts. Further faecal samples of diarrheic calves collected by high culture sterile swab were streaked on Nutrient agar plates by standard procedure to obtain isolated colonies of bacteria. To isolate Escherichia coli and *Salmonella* spp., the isolated colonies were subcultured and incubated on Eosin Methylene blue agar (EMB) and XLD agar for 24 and 48 hours, respectively. Thirty bovine calves were included in the study, and it was discovered that they were positive for *Salmonella* spp. and negative for endoparasites.

2.1 Clinical examination
Complete physical examination was performed on all animals and vital parameters were recorded including rectal temperature, heart rate and respiratory rate Radostits *et al.* (2007) [14]. Walker *et al.* (1998) [21] determined the clinical scores (0-3) for dehydration, clinical depression, and faecal consistency.

2.2 Collection of sample
After clinical examination of animal's blood samples of cattle were collected from jugular vein of diarrhoic calves as well from control group, aseptically in ethylene diamine tetra acetic acid (EDTA) coated vacutainers for haematological examination.

2.3 Haematological parameters
Hematological measures include mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (McHc), packed cell volume (PCV), total leukocyte count (TLC), total erythrocyte count (TEC), differential leucocyte count (DLC), and mean corpuscular volume (MCV). According to standard haematological procedures as stated in Schalm's Veterinary Haematology (Jain, 1986) [22], the analyses were carried out.

2.4 Biochemical parameters
Every animal had 5 ml of blood drawn into a clot activator tube from its jugular vein while safety measures were implemented to avoid hemolysis. The serum was isolated and kept at -20 °C until biochemical analysis after the blood was centrifuged for 5 minutes at 4500 rpm after it had been allowed to clot. IDEXX Vet Test Chemistry Analyzer (IDEXX Laboratories, West brook, Maine, USA) utilized kits from IDEXX Laboratories, USA to measure serum total protein, albumin, globulins, A:G ratio, and glucose in accordance with the manufacturer's prescribed procedure.
Electrolyte estimation of serum samples for sodium, potassium, chloride and bicarbonate were carried out by CKK lyte electrolyte analyzers (ARK diagnostics Bangalore pvt. Limited). The Principles, reagents required, calibration, operating procedures, calculation and precautions were according to the guidelines provided in the operator's manual.

2.5 Statistical analysis
The means and standard errors (SE) of the values were reported. The t-Test was used for the statistical analysis, with a significance level test of 95% ($p \leq 0.05$).

3. Results and Discussion
Nine (30%) of the diarrhoeic calves had a clinical score of "1," indicating they had pasty feces, mild depression, had not suckled vigorously, mild dehydration, eyes that did not recede into their orbits, and slight looseness in their skin. Fifteen (50%) of the diarrheal calves had a clinical score of "2," indicating they had semi-liquid feces, significant depression, weak or erratic suckling, moderate dehydration, eyes that slightly receded into orbit, and skin tenting times longer than three seconds. Six (20%) of the calves who had diarrheal stools had watery stools, were severely dehydrated, couldn't stand and nurse, and had a clinical score of "3." All healthy calves taken for study were showing vigorous suckling reflex, bright eyes, pliable skin and normal hydration status and scored as "0" shown in table 1.
When comparing diarrheal calves to the control group shown in Table 2, we found no significant difference ($p>0.05$) in rectal temperature, respiration, or heart rates. These results are consistent with those of Sharma (2013) [11] and Maurya *et al.* (2016) [13], who also found non-significant changes in the vital parameters in diarrheal calves.
Haematology revealed highly significant ($p<0.01$) increase in Hb, PCV, TEC and TLC as well as significant ($p<0.01$) neutroplilia and lymphopenia in diarrhoeic calves in comparison to control group shown in table 3 (Sharma 2013; Bashir *et al.*, 2015; Shekhar *et al.*, 2017) [11, 2, 18]. In the current study, haemo-concentration associated with hypovolemia and dehydration caused an increase in Hb, PCV, and TEC levels in the diarrheal calves. This is because diarrhea causes an excessive loss of intestinal fluid, which can lead to severe dehydration (Radostits *et al.*, 2007) [14]. Increased TLC attributed to defence mechanism against infection and it was found that affected calves were infected with both *E. coli, Salmonella* spp. or mixed infection of these two bacterial pathogens in intestines probably responsible for leucocytosis Eddy and Pincent (2004) [8]. Partially generalized tissue dehydration also leads to leucocytosis Bukhari (2002) [6]. As a result of an enteropathogenic bacterial infection, diarrheal calves had significant neutrophilia and concurrent lymphopenia (Brar *et al*., 2015; Malik *et al*., 2013) [3, 11].
The results of the biochemical analysis of serum indicate a highly significant ($p<0.01$) increase in total protein, albumin, globulin, and A:G, while table 4 and nearly similar observations made by Sharma (2013) [11], Singh *et al.* (2014) [19], and Shekhar *et al.* (2017) [18] show a significant ($p<0.01$) hypoglycaemia in diarrhoeic calves compared to control ones. Hypervolaemia, hemoconcentration, decreased glomerular filtration rate, and hyperproteinemia, hyperalbuminemia, and hyperglobulinemia are the findings (Walker *et al.*, 1998; Malik *et al.*, 2013) [21, 11]. Hyperalbuminemia is indicative of hazardous acute tissue dehydration (Gupta, 2006) [9]. Hypoglycemia in diarrheal calves has been associated to impaired intestinal absorption of glucose, inappetence/anorexia, and a reduced rate of lactic acid conversion to glucose.
Table 5 shows that the serum electrolyte profile revealed a significant ($p<0.01$) increase in potassium concentration and a significant ($p<0.01$) decrease in sodium, chloride, and bicarbonate. Our results align with the findings of Shekhar *et al.* (2017) [18], Bashir *et al.* (2015) [2], and Singh *et al.* (2014) [19]. In addition to cellular damage, hyperkalaemia is caused by increased tubular reabsorption of K+ ions in response to acidosis and increased renal retention of K+ ions (Radostits *et al*., 2007; Singh *et al*., 2014) [14, 19]. Due to the diffusion of H+ ions from acid plasma into the intracellular fluid compartment

and the diffusion of K+ ions into the plasma to maintain electroneutrality, partially hyperkalemia arises (Tasker, 1991 and Bouda *et al.*, 1997) [5, 4]. When animals have diarrhea, their injured intestinal villus cells release too many sodium ions into the circulation, which leads to hyponatraemia (Radostits *et al.*, 2007) [14]. According to Radostits *et al.* (2007) [14], hypochloraemia is brought on by a decrease in the amount of chloride ions lost from the intestines as well as a breakdown in the villus' ability to reabsorb gastric H+ and Cl- ions. When colloidal protein particles diffuse into the tissues of diarrheal calves, there is a decrease in serum bicarbonate (HCO3-) as a result of enhanced capillary permeability (Radostits *et al.*, 2010) [15].

Table 1: Clinical score (0-3 basis) of healthy control and diarrhoeic calves

Score	Control calves	Diarrhoeic calves
0	10	0
1	0	9 (30%)
2	0	15 (50%)
3	0	6 (20%)

Table 2: Mean ± SE values of physiological parameters of diarrhoeic and control calves

S. No.	Parameters	Healthy calves (n=10)	Diarrhoeic calves (n=30)	Statistical analysis (T test)
1.	Rectal temperature (ºF)	101.19±0.17	101.75±0.23	NS
2.	Heart rate	113.2±0.57	114.3±0.38	NS
3.	Respiration rate	18.3±0.45	19.13±0.33	NS

NS=Non significant (p>.05)

Table 3: Mean ± SE values of haematological parameters of diarrhoeic and healthy control calves

S. No.	Parameters	Healthy calves (n=10)	Diarrhoeic calves (n=30)	Statistical analysis (T test)
1.	Hb (g/dl)	10.46±0.18	11.62±0.11	**
2.	PCV (%)	38.8±0.89	46.31±0.36	**
3.	TEC (10^6/µl)	7.89±0.15	8.91±0.11	**
4.	MCV (fl)	48.22±1.3	49.16±0.46	NS
5.	MCH (pg)	13.26±0.47	14.16±0.19	NS
6.	MCHC (g/dl)	27.25±0.75	28.10±0.48	NS
7.	TLC (10^3/µl)	10.28±0.11	13.53±0.19	**
8.	DLC			
	(i) Neutrophils (%)	37.5±0.91	44.87±0.53	**
	(ii) Eosinophils (%)	1.6±0.31	0.97±0.17	NS
	(iii) Basophils (%)	0.4±0.16	0.437±0.09	NS
	(iv) Lymphocytes (%)	60.6±1.06	53.7±0.59	**

**=p<.01 *=p<.05 NS=Non significant (p>.05)

Table 4: Mean ± SE values of serum biochemical parameters of diarrhoeic and control calves

S. No.	Parameters	Healthy calves (n=10)	Diarrhoeic calves (n=30)	Statistical analysis (T test)
1.	Total protein(g/dl)	6.53±0.12	7.85±0.11	**
2.	Albumin(g/dl)	2.99±0.07	3.67±0.06	**
3.	Globulin(g/dl)	3.56±0.05	4.17±0.06	**
4.	A:G ratio	0.84±0.01	0.88±0.01	**
5.	Glucose(mg/dl)	60.1±1.59	46.37±0.97	**

**=p<.01 *=p<.05 NS=Non significant (p>.05)

Table 5: Mean ± SE values of serum electrolyte parameters of diarrhoeic and control calves

S. No.	Parameters	Healthy calves (n=10)	Diarrhoeic calves (n=30)	Statistical analysis (T test)
1.	Serum sodium(mmol/L)	139.96±0.78	127.02±0.32	**
2.	Serum potassium (mmol/L)	4.45±0.06	5.48±0.04	**
3.	Serum chloride (mmol/L)	101.21±1.25	96.51±0.43	**
4.	Serum bicarbonate (mEq/L)	23.94±0.23	16.12±0.31	**

**=p<.01 *=p<.05 NS=Non significant (p>.05)

Conclusion

Present study concludes that diarrhoea is a dreadful disease of cattle calves. Clinically disease is characterized by lethargyness, dehydration and in severe cases recumbency. Haematology analysis of the diarrheal calves showed an increase in Hb, PCV, TEC, TLC, neutrophilia, and lymphopenia. Serum biochemical analysis revealed hyperproteinemia, hyperalbuminemia, hyperglobulinemia and hypoglycemia in diarrhoeic calves. Serum electrolyte profile revealed hyperkalemia, hyponatraemia, hypochloraemia and decreased bicarbonate in diarrhoeic calves. This study provided additional insight into the pathophysiology of diarrhoea. Our results could have clinical significance in the implementation of good management and early treatment strategy in diarrhoeic cattle calves.

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