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Alterations in haematological and biochemical parameters in diarrhoeic buffalo calves

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Abstract
To identify diagnostic/prognostic indicators for infectious diarrhoea in buffalo calves, blood and faecal samples from 30 diarrhoeic calves were collected. Six healthy buffalo calves' samples were collected and constituted group I. Blood was collected into two separate vials having EDTA for estimation of hemoglobin (Hb), packed cell volume (PCV), red blood cell count (RBC), total leucocytic count (TLC), and differential leucocytic count (DLC) and heparin for separation of plasma for biochemical analysis (Total plasma protein, albumin, globulin and albumin to globulin ratio). Faecal samples' screening was done by floatation method and grouping was done on the basis of parasitological findings. Mean values of PCV, TLC, total protein and albumin were found to be increased significantly (p<0.05) indicating haemoconcentration due to diarrhoea and parasitic infestation. Slight increment in lymphocytes was also observed in various diarrhoeal groups.

Keywords: Buffalo calf, diarrhoea, haematological, biochemical

Introduction
Livestock is an integral part of the agricultural production system in Indian economy as well as in socio-economic development of millions of rural household. Diarrhoea, being the leading cause of death in calves aged less than six months, is a major source of economic loss to the cattle industry. It can be caused by a variety of pathogens including bacteria, viruses, protozoa and intestinal parasites.

Several entero-pathogens associated with calf diarrhoea and their relative prevalence varies geographically but the most prevalent infections in most areas are enterotoxigenic *Escherichia coli*, *Salmonella typhimurium*, *Clostridium perfringens*, bovine rotavirus (BRV), coronavirus (BCV) and the protozoan parasite *Cryptosporidium parvum* (Bartels *et al*., 2010; Izzo *et al*., 2011) [4, 12]. E. coli K99 causes diarrhoea only during the first week of life, BRV, BCV, *C. parvum* and Salmonella also affect older calves (Bazeley, 2003; Foster and Smith, 2009) [5, 9]. It causes varying degree of dehydration, gastroenteritis, body fluid loss and various body fluid changes as well as alterations in various serum metabolites. These pathogens cause specific enteric infections, resulting in secretory or Malabsorption diarrhoea, along with inflammation of the intestinal epithelium resulting in rapid loss of weight, dehydration, loss of electrolytes and severe disturbance of acid base and electrolyte balance and death. Cases of calf diarrhoea are commonly found associated with more than one of these agents and the cause of most outbreaks is also multifactorial. Dehydration resulting from diarrhoea has been reported as the major cause of death (Edwards and William, 1972) [7]. Loss of 10% body water results in serious metabolic disturbances resulting in loss of water, sodium, potassium, chloride, bicarbonate and also haematological and other biochemical parameters. Shortening of RBC survival has been reported (Seifi *et al*., 2006) [20] in diarrhoeic cattle calves. Such variations in response to diarrhoea can be used to monitor the response to therapy, to gage the severity of an illness or as a starting point for formulating a list of differential diagnosis. There are a number of parameters that are known or proposed to be useful in measuring calf predisposition to morbidity or mortality; some of them are total red blood cell count (tRBC), haemoglobin (Hb) and packed cell volume (PCV). Also the gastrointestinal infections have been reported to produce different patterns of intracellular and RBCs' membrane damage. It is unclear whether his is a protective reaction or simply a biochemical reaction that occurs with excessive oxidative stress (Lykkesfeldt and Svendsen, 2006) [14].

Materials and Methods Materials

I Animals

Under this study, thirty buffalo calves (1-6 months of age, male and female) suffering from diarrhoea either referred to the TVCC, or in the nearby villages of Hisar district were used for sampling**.** Samples were collected after 3-5 days of illness**.** In addition to these, six healthy calves from LUVAS farm were kept as control and constituted Group-I.

Clinical examination

The most prominent clinical signs among the buffalo with diarrhoea were mild to severe diarrhoea, depression, dullness and depraved appetite. The animals were weak and reluctant to move. Based on clinical signs, faecal consistency and appearance, the type of diarrhoea is presented in Table-1.

Table 1: Various types of diarrhoea and clinical observations associated with it

Group No.	Appetite	Diarrhoea (Faecal consistency)	Microscopic Faecal examination		
			Balantidium coli	***Strongyle/strongyloid* spp**	***Eimeria* spp.**
1	Normal	Semi solid and greenish coloured	-ve	-ve	-ve
2	Slight anorectic	Loose and whitish yellow	+ve	-ve	-ve
3	Slight anorectic	Loose with some undigested ingesta	-ve	+ve	-ve
4	Anorectic	Bloody diarrhoea	-ve	-ve	+ve
5	Mild to severe anorexia	Fluidy with greenish discolouration	-ve	-ve	-ve

The animals were screened for presence of parasitic ova by floatation technique. The animals which were found positive for the presence of *Balantidium coli*, *Strongyle* and *Strongyloid* spp., *Eimeria* spp., constituted as Groups II, III and IV respectively as tabulated in Table-2. Animals having profuse diarrhoea but faecal samples were negative for parasitic ova constituted Group-V.

Table 2: Various groups under study and number of animals in each group

Groups	Number of animals in each group	Faecal samples positive for
Group I (Control)	6	None
Group II	3	*Balantidium coli*
Group III	16	*Strongyle/strongyloid* spp.
Group IV	3	*Eimeria* spp.
Group V	8	Non parasitic diarrhoea

II. Collection of blood samples

Approximately 5 ml blood was collected from jugular vein using 10 ml disposable syringe aseptically. One ml of blood was poured into vial containing ethylenediamine-tetra-acetic acid (EDTA) for hematological examination; 4 ml blood was poured in centrifuge tube containing heparin for separation of plasma and preparation of haemolysate. Faecal samples were also collected in sterile containers to avoid contamination and were subjected to parasitological examination. Haematology was done as early as possible. For separation of plasma and preparation of haemolysate to measure oxidative stress indices, the blood samples collected in centrifuge tube containing heparin were centrifuged at 3000rpm for 10 minutes; the plasma was separated in aliquots and buffy coat layer was removed to harvest the red blood cells. After that, red blood cells were washed thrice in an ice-cold normal saline solution (NSS). A part of RBC pellet was diluted with ice-cold distilled water in 1:1 dilution for the preparation of stock haemolysate. The plasma and haemolysate were stored in liquid nitrogen in aliquots till further analysis for estimation of biochemical parameters.

Methods

The various parameters analysed were as follows

(1) Haematological analysis

The blood samples collected in EDTA vials were used for haematological examination using fully automated Haematology cell counter (MS4s, Melet Schlosing Lab.). The erythrocytic indices measured were haemoglobin (Hb), total erythrocyte count (TEC), packed cell volume (PCV). The leucocytic indices measured were total leucocyte count (TLC), lymphocytes (L), monocytes (M), neutrophils (N), eosinophils (E) and basophils (B).

(2) Microscopic examination of faecal samples

It was done by floatation method to detect the presence of parasitic ova as described by Soulsby (2007) [24]. Faecal floatation procedures are based on differences in specific gravity of parasite eggs, cysts and larvae of faecal debris. Most parasite eggs have a specific gravity between 1.1 and 1.2g/ml whereas tap water's density is only slightly higher than 1g/ml. About 4gm of diarrhoeic faeces were taken and homogenised using pestle and mortar. Saturated sodium chloride solution (15ml) was added in the glass tube and filled up to brim. A slide was kept on the top and left undisturbed for 20-25min. and was removed in the horizontal position. Coverslip was applied quickly and examined microscopically for parasitic ova such as Strongyles, Strongyloides, Ascarids, Eimeria, Balantidium, oxyurids, trichuris and cestode ova.

(3) Statistical analysis

The mean of different parameters was subjected to statistical analysis as outlined by Snedecor and Cochran (1994) [22]. Difference of significance in variables among five groups was compared with the help of one-way analysis of variance (ANOVA) in SPSS computer software.

Results

Haematological values

Haemoglobin (Hb), Packed Cell Volume (PCV) and Red Blood Cells (RBC) count

Mean value of PCV was found to be increased significantly (p<0.05) in all groups as compared to healthy control group except group IV where it decreased (Table-3).

Table 3: Changes in haematological values (Mean ± SE) due to diarrhoea in buffalo calves.

Parameters	Group I (n=6)	Diarrhoeic calves (n=30)			
		Group II (n=3)	Group III (n=16)	Group IV (n=3)	Group V (n=8)
Hb (g/dl)	8.68±0.40^{a}	8.73±0.98^{a}	9.04±0.40^{a}	6.56±1.24^{a}	9.67±0.48^{a}
PCV (%)	28.38±0.27^{ab}	29.56±1.49^{ab}	30.83±0.76^{b}	26.46±1.12^{a}	31.41±1.01^{b}
RBCs(10^{6}/ µl)	6.53±0.24	6.78±1.31	7.11±0.39	4.94±1.19	7.92±0.38

Values with common superscripts do not differ significantly between groups at p<0.05.
Gp.-I, Healthy control; Gp-II, *Balantidium coli* positive; Gp-II, *Strongyle/Strongyloid* spp. positive; Gp-IV, *Eimeria* spp. positive; Gp-V, Non parasitic diarrhoeal group

Total and Differential Leucocytic Count

The mean value of total leucocytic count was found to be increased significantly (p<0.05) in groups III and V as compared to healthy control group\but decreased significantly in group IV(Table 3).

Table 4: Changes in total leucocytic and differential leucocytic count (TLC and DLC) values (Mean ±SE) due to diarrhoea in buffalo calves

Parameters	Group I (n=6)	Diarrhoeic calves (n=30)			
		Group II (n=3)	Group III (n=16)	Group IV (n=3)	Group V (n=8)
TLC (10^{3}/µl)	7.79±0.99^{ab}	8.07±1.66^{ab}	9.07±0.40^{b}	5.79±1.40^{a}	9.38±0.32^{b}
L (%)	47.26±3.96	51.96±2.74	53.68±2.81	44.90±5	30.62±0.33
N (%)	49.95±4.39	45.70±6.35	42.25±2.94	50.33±5.25	56.68±4.59
M (%)	2.20±0.49	2.36±0.31	2.60±0.10	3.26±0.55	3.06±0.33
B (%)	0.18±0.06	0.36±0.14	0.16±0.02	0.13±0.03	0.18±0.05
E (%)	0.40±0.32	0.93±0.20	0.95±0.169	1.43±0.42	0.68±0.17

Values with common superscripts do not differ significantly between groups at p<0.05. Gp-I, Healthy control; Gp-II, *Balantidium coli* positive; Gp-III, *Strongyle/Strongyloid* spp. positive; Gp-IV, *Eimeria* spp. positive; Gp-V, Non parasitic diarrhoeal group

Total plasma protein, albumin, globulin and albumin to globulin ratio

Mean values of total plasma proteins increased significantly (p<0.05) in groups II to V (Table- 5).

There was significant increase in mean values of albumin in diarrhoeic groups as compared to control group (Table- 5).

Table 5: Changes due to diarrhoea in buffalo calves in total plasma protein, albumin, globulin and albumin to globulin ratio (Mean ±SE)

Parameters	Group I (n=6)	Diarrhoeic animals (n=30)			
		Group II (n=3)	Group III (n=16)	Group IV (n=3)	Group V (n=8)
TP(g/dl)	6.69±0.16^{a}	7.82±0.07^{b}	7.74±0.56^{b}	7.72±0.20^{b}	7.86±0.62^{b}
Albumin(g/dl)	3.06±0.21^{a}	3.94±0.04^{b}	3.90±0.61^{b}	3.84±0.09^{b}	3.92±0.62^{b}
Globulin(g/dl)	3.63±0.17	3.87±0.06	3.83±0.54	3.87±0.11	3.94±0.66
A:G	0.84±0.93	1.01±0.01	1.06±0.36	0.99±0.01	1.03±0.29

Values with common superscripts do not differ significantly between groups at p<0.05. Gp-I, Healthy control; Gp-II, *Balantidium coli* positive; Gp-III, *Strongyle/Strongyloid* spp. positive; Gp-IV, *Eimeria* spp. positive; Gp-V, Non parasitic diarrhoeal group

Discussion

Mean levels of haemoglobin, PCV and RBCs were found to be increased in diarrhoeal groups as compared to healthy control group except the group which was affected with Eimeriosis while significant increase was only in PCV. Similarly, a number of workers (Groutides and Michell, 1990; Bali *et al*., 1999; Anwar *et al*., 1999) [11, 3, 2] have reported increased levels of Hb, PCV and RBC count in various types of diarrhoeas. The increase in haematological values in groups II, III and V was due to haemoconcentration associated with dehydration. These findings were in concurrence of findings by Roy *et al*., 2009 [19] and Brar *et al*., 2015 [6] reported PCV as indicator of dehydration. Thus, estimation of PCV is of utmost importance to monitor hydration status of animal and is a sensitive indicator for assessing the severity of dehydration. Significant elevation of TEC in scouring calves has also been reported by Sridhar *et al*., 1988 [25]. Leucocytosis in these groups might be due to normal reaction of body defence mechanism against infection and also due to dehydration resulting into haemoconcentration.

Differential leucocyte counts, which are the suggestive of infectious agents of different origin, revealed no significant changes in these values except slight decrease in lymphocytes (%) and increase in neutrophils (%) in group V i.e. non parasitic diarrhoeal group.

The values of PCV and TLC were found to be reduced significantly in Eimeria infected Group IV as shown in Tables 3 and 4. The decrease in different haematological values has been reported by several workers, for instance, Shommein and Osman, 1980 [21] observed a considerable fall in TEC in coccidiosis. The results of the present investigation are in accordance with the findings of these workers. This considerable decrease in TEC could be due to the blood loss from the haemorrhagic intestinal mucosa and bloody diarrhoea. Hb and PCV values decreased because both are dependent upon erythrocytic count and with its decrease, the haemoglobin and packed cell percentage has decreased proportionally. Similar observations are made by Svanbaev and Gorbunova, 1969 [26] and Fitzgerald and Mansfield, 1984 [8]. Similar to the present investigation, the increase in TLC because of their phagocytic role have been

reported by Rama *et al*., 1978 [18]. No significant changes were observed in differential leucocytic count. Among DLC, an increase in neutrophils and a decrease in the eosinophils count along with marked lymphopenia was found. Similar pattern has been observed by Gretillat, 1976 [10]. This increase in eosinophils may be related to its important role in neutralizing histamine, which is released from damaged intestinal cells and foreign substances, detoxified by eosinophils and then phagocytized.

The protein is the most abundant component of plasma. It has been suggested that (Anderson and Anderson, 2002) [1] virtually all diseases affect the proteins found in serum. A significant increase in total plasma protein, albumin and slight increase in albumin to globulin ratio in diarrhoeic calves were apparently due to associated dehydration. Tenant *et al*., 1972 [27]; and Walker *et al*., 1998 [31] observed the significant increase in both PCV and total plasma protein, which indicates hypovolemia, haemo-concentration and reduced glomerular filtration rate.

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