The Pharma Innovation Journal 2020; 9(1): 10-13

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(1): 10-13

www.thepharmajournal.com
Received: 06-11-2019
Accepted: 10-12-2019

Sarvan Kumar
Department of Veterinary Pathology, Faculty of Veterinary and Animal Sciences, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Utter, Pradesh, India

KK Jakhar
Department of Veterinary Pathology, College of Veterinary Sciences, LUVAS, Hisar, Haryana, India

Corresponding Author:
Sarvan Kumar
Department of Veterinary Pathology, Faculty of Veterinary and Animal Sciences, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Utter, Pradesh, India

Haematological, biochemical and oxidative stress parameter as prognostic indicators in calf diarrhoea

Sarvan Kumar and KK Jakhar

Abstract
The objective of this study was to evaluate the value of haematological, biochemical constituents and oxidative stress parameters for predicting the survival of diarrhoeic calves. Investigation was carried out on 31 cow calves including 6 healthy and 25 calves affected with diarrheoa. The haematological studies evident that there was significant decrease in value of haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC). Erythrocyte indices i.e. Mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were significantly decreased in diarrhoeic calves. There was significant increase in total leukocytic count (TLC) and in value of absolute lymphocytes in diarrhoeic calves when compared with the corresponding values of healthy calves. Serum biochemical studies revealed that there were significant increase in values of serum total protein, albumin, globulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LD) however there was non significant decrease in albumin: globulin ratio in diarrhoeic calves. Studies of oxidative stress parameters showed that there was significant decreases in activity of superoxide dismutase (SOD), glutathione-s- transferase (GST) and glutathione reductase (GR) whereas significant increase in activity of catalase in diarrhoeic calves when compare with apparently healthy calves; respectively.

Keywords: Calf diarrhoea, hematological examination, serum biochemical examination, oxidative stress

Introduction
The effective development of any livestock industry depends upon prevention and control of diseases among these animals. Diseases in these animals cause heavy economic losses in milk, meat and wool industry. Bacterial organisms and parasites play an important role in diseases causing heavy morbidity and mortality as they are important etiological agents causing gastroenteritis in calves (Singh *et al*., 2009 and Singh *et al.,* 2014) [21, 22]. Some of the diseases are Johne's disease (Paratuberclosis), colibacillosis, enterotoxemia, botulism, vibrio dysentery, salmonellosis, *Clostridium prefringens* type- B, C (enterotoxaemia) and type-D, bloat and diarrhoea. Gastrointestinal parasites are also considered as a major challenge for the health and the welfare of animals. Parasitism, especially by helminthic parasites, impairs health by causing inappetance, diarrhoea, anaemia and, in severe cases, death. The nematode parasite, *Toxocera vitulorum* is singly the most important nematode pathogen of ruminants throughout the India. Although considered to be the greatest problem in the tropics/subtropics, this parasite has shown a definite trend of increasing prevalence and economic importance in organized and unorganized farms. Other internal parasitic infections seen in bovine are adult tapeworms (*Moniezia* spp.) and several intermediate stages (Metacestodes) of tapeworms (*Taenia* spp.). Calf mortality leads to losses in milk yield and also reduces the overall output of livestock production (Singh *et al*., 2009) [21].
Diarrhoea remains the leading cause of mortality in dairy and beef calves (Constable, 2004) [8]. The traditional indices such as clinical signs and faecal consistency give poor guidance to correct the decisive pathological disturbances (Michell *et al*., 1992) [17]. Pare *et al*., (1993) [19] showed that haematocrit and total protein at or shortly after birth may have important prognostic value in evaluating risk of calf diarrhoea. Others have also shown that the blood urea concentration has the best prognostic value and conclude that, by measuring two other parameters (The haematocrit and the blood chloride, Cl, concentration), they were able to classify the calves into two distinct dead or survivor groups with 80% accuracy (Fayet and Overwater, 1978) [11]. During diagnostic procedure it is very useful to compare the values obtained from diarrhoea calves with normal values in healthy calves.

In the current study, we have used haematological, biochemical and oxidative stress parameters to predict the fate of diarrhoeic calves. This is of paramount importance in treatment strategies as measuring haematological, biochemical and oxidative stress values of the blood provide veterinarians with useful information in a matter of few hours.

Materials and Methods

Ethical approval

The study was conducted after the approval of the Institutional Animal Ethics Committee.

Collection of sample

Proposed study was conducted on 31 calves (25 diarrhoeic calves and 6 apprantely healthy calves). Blood samples were collected from 25 calves which showed signs and symptoms of diarrhoea, which were brought to Teaching Veterinary Clinical Complex, LUVAS, Hisar and nearby organized/ unorganized farms of Hisar regions and 6 apparently healthy calves which have normal physiological parameters like temperature, pulse rate and respiration rate and normal behavior to feeing and others animals. A complete clinical evaluation was performed before collection of samples for investigation of various parameters. About 5ml of blood was collected through jugular vein aseptically in the clean sterilized glass vials containing anticoagulant. In addition to this 10 ml of blood was collected without anticoagulant in clean and sterilized glass vials and allowed to clot at room temperature to separate serum. Samples were shifted immediately to the laboratory, to avoid any deleterious effect. The serum samples were stored in deep freeze (-20 ºC) in vials till further use.

Hematological examination

Haematological studies viz; haemoglobin (Hb), packed cell volume (PCV), total erythrocytic count (TEC), total leukocyte count (TLC), differential leukocytic count (DLC), absolute leukocytic count and erythrocytic indices namely Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin and Mean Corpuscular Haemoglobin Concentration (MCHC) were estimated following the method of Benjamin (2013) [3] with in the six hours of blood collection.

Serum biochemical examination

Serum biochemical parameters were estimated by using Chem-5 (Erba Mannheim) chemistry analyzer using standard diagnostic kits of Erba (Transasia). The levels of the following serum constituents viz. total protein (Tietz, 1986b) [24], albumin (Doumas *et al*., 1972) [9], ALT (Wroblewski and La Due 1956), AST (Tietz, 1986a) [25], ALP (Tietz, 1986b) [24] and LDH (Tietz, 1986b) [24] were measured. The gamma-globulin levels and albumin: globulin ratio were calculated

Oxidative stress parameters

The plasma catalase activity was measured as per method described by the Aebi (1983) [1]. In brief 20 µl of 1% erythrocyte lysate was incubated in 1.0 ml of 30 mM H_2O_2 at 37^0C and decrease in absorbance was noted every 10 sec interval for one min. at 240 nm in a UV spectrophotometer (Yorco Digital Spectrometer). The catalase activity was expressed as µM of H_2O_2 decomposed/min/mg Hb using 36 as molar extinction coefficient of H_2O_2. The activity of SOD in 1% erythrocyte lysate was determined by the method of Marklund and Marklund (1974) [16]. The assay was based on the ability of SOD to exhibit the auto oxidation of pyrogallol in presence of ethylene diamine tetra acetic acid (EDTA). The values were expressed as units/mg Hb. Glutathione reductase (GR) activity was assayed according to the method of Calberg and Mannervik (1985) [7]. NADPH oxidation was monitored at pH 7.4 and 30 ^{0}C, and enzyme activity was expressed in terms of micromoles of NADPH oxidized per gram of Hb per min. Glutathione-s-transferase (GST) activity with 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate was measured according to Habig *et al*. (1974) [12]. Formation of the S-conjugate was followed by its absorbance at 340 nm. The activity was expressed in terms of micromoles of CDNB conjugated per gm of Hb/min.

Statistical analysis

Analyses of the data were undertaken by Student's t-test using SPSS 17.0 software (IBM Corporation, New York, USA) as per the Snedecor and Cochran (1994) [23].

Results and Discussion

The results of haematological studies are presented in Table 1(a-c). It is evident that there was significant ($P \leq 0.05$) decrease in value of Hb (9.1 ± 0.54), PCV (24.9 ± 1.31), TEC (6.2 ± 0.36) and significant increase in TLC (17.4 ± 2.42) in affected with diarrhoea, when compared with the corresponding values of healthy calves. There was significant decrease in the values of erythrocyte indices i.e. MCH (13.6 ± 0.29), MCHC (32.9 ± 0.71) and MCV (35.7 ± 1.29) in diarrhoeic cow calves. There was significant ($P \leq 0.05$) increase in absolute values of lymphocyte (12.88 ± 2.21) in cases diarrhoeic calves whereas non significant increase in value of neutrophils (3.756 ± 0.49), monocytes (0.53 ± 0.07) and eosinophils (0.42 ± 0.05) was observed when compared with the value of apparently healthy cow calves; respectively.

Decrease in value of Hb, PCV and TEC might be due to malabsorpation of principle nutrients which were required for haemopoiesis, presence of strongyle infection, which has been recognized as active blood sucker in stomach and infection of enterohaemorrhagic *E. coli* which is establish aetiology of haemorrhagic enteritis. Significant increase in absolute values of lymphocyte in diarrhoeic cow calves and neutrophils and lymphocyte in diarrhoeic buffalo calves due to response of cellular body defense mechanism to various aetiological agents like bacteria, bacterial toxin and virus (Jubb, 1993) [13]. These findings are in consent with finding of Alsaad *et al.*, 2012 [2]; Malik *et al.*, 2013 [15]; Botezatu *et al.*; 2014 [4]; Singh *et al.*; 2014 [22] and Brar *et al.*; 2015 [5].

The results of serum biochemical studies of apparently healthy and diarrhoeic cow calves are depicted in table 2 (a-b). It is evident from serum biochemical studies that there were significant ($P \leq 0.05$) increase in values of serum total protein (9.6 ± 0.48), albumin (4.9 ± 0.28) and globulin (4.7 ± 0.24) however there was non significant ($P \leq 0.05$) decrease in albumin: globulin ratio (1.03 ± 0.03) in diarrhoeic calves. A significant increase ($P \leq 0.05$) in activities of ALT (22.8 ± 0.98), AST (57 ± 3.38), ALP (69.6 ± 2.17) and LD (286 ± 14.40) were observed in calves diarrheoa when compared with the value of apparently healthy calves respectively.

Increase in values of total protein, serum albumin and serum globulin might be due to haeomoconcentration however decrease in albumin: globulin ratio due to increase in fraction of relative globulin in diarrhoeic cow calves and buffalo calves (Duncan, 1994; Kaneko, 1997) [10, 14]. These finding

more or less similar to finding of Malik *et al.* (2013) [15], Singh *et al.* (2014) [22] and Brar *et al.* (2015) [5]. Immunoglobulins are effectively produced by body defense mechanisms in response to infection. Increase in activities of ALT, AST, ALP and LDH might be due to pathological lesions like cell necrosis, damage to cell membrane and change in permeability of cell membrane of liver, intestine, damage to cells of lining epithelium of intestine (mucous membrane) and muscle fibers, increase in activity of osteoclasts and osteoblasts and cardiac muscle. Damage or increase in permeability of cell membrane leads to leakage of these intracellular enzymes and comes to plasma (Benjamin, 2013; Kaneko, 1997) [3].

In diarrhoeic cow calves, observation of oxidative stress parameters revealed that there were significant ($P \leq 0.05$) decrease in activity of SOD (5.36 ± 0.35), GST (32.45 ± 0.99) and GR (0.97 ± .063) where as significant ($P \leq 0.05$) increase in activity of catalase (73.4 ± 1.74) as depicted in table 3.

Observation of oxidative stress parameters revealed that there were significant decreases in activity of superoxide dismutase, glutathione s transferase and glutathione reductase whereas significant increase in activity of catalase in diarrhoeic cow calves as well as in case of buffalo calves. These finding were in consent with the finding of Sharma *et al.* (2011) [20]. Oxygen derived free radical are produced during normal metabolic processes in reduction-oxidation reaction in normal respiration and oxygen dependent effective killing of bacterial agent by (myeloperoxidase dependent/H_2O_2-MOP- Halide System and myeloperoxidase independent) in diarrhoeic/diseased animals. Superoxide dismutase, glutathione and glutathione peroxidase are utilize in effective neutralization of these free radicals so their concentration were decreased in diarrhoeic/diseased animals (Vegad and Katiyar, 2008) [26] whereas increase in activity of catalase might be due to marked reduced in activity of catalase enzyme with increase in temperature (Morgulis *et al.*, 1926) [18]. In present study, there was significant increase in temperature in diarrhoeic calves so in vivo activity of catalase was decreased and in vitro increase in concentration.

Based on the results of this study it may be concluded that significant decrease in values of Hb, PCV, TEC and leucocytosis due to absolute neutrophilia and absolute lymphocytosis in diarrhoeic calves. Significant increase in values of serum total protein, albumin and globulin were noticed in diarrhoeic calves might be due to haemoconcentration. Significant increase in activities of serum ALT, AST, ALP and LDH in diarrhoeic buffalo calves are indicating that there might be some cellular level damage in tissues of major organs like liver, heart, kidney and musculature of buffalo calves. Significant decrease in values of SOD, GST and GR in diarrhoeic buffalo calves might be due to utilization of these enzymes to neutralize free produced during diarrhoeic state.

Table 1(a): Haemoglobin (Hb), packed cell volume (PCV), total erythrocytic count (TEC) and total leukocytic count (TLC) in apparently healthy and diarrhoeic calves

Groups	Hb (g/dl)	PCV (Per cent)	TEC (10^6/µl)	TLC (10^3/µl)
Apparently healthy calves (n=6)	12.7^a± 0.41	37.7^a± 0.81	8.1^a ± 0.49	7.0^a ± 0.47
Diarrhoeic calves (n=25)	9.1^b± 0.54	24.9^b± 1.31	6.2^b ± 0.36	17.4^b ± 2.42

Mean ± S.E., Different superscripts in columns $P \leq 0.05$.

Table 1(b): Erythrocyte indices: mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) in apparently healthy and diarrhoeic buffalo calves

Groups	MCH (g/dl))	MCHC (pg)	MCV (fl)
Apparently healthy calves (n=6)	15.8 ± 0.51	33.7 ± 0.43	47^a ± 1.78
Diarrhoeic calves (n=25)	13.6 ± 0.29	32.9 ± 0.71	35.7^b ± 1.29

Mean ± S.E., Different superscripts in columns $P \leq 0.05$.

Table 1(c): Absolute leukocytic count in apparently healthy and diarrhoeic cow calves.

Groups	Neutrophil (10^3/µl)	Lymphocyte (10^3/µl)	Eosinophil (10^3/µl)	Monocyte (10^3/µl)
Apparently healthy calves (n=6)	2.187 ± 0.14	4.512^a ± 0.36	0.262 ± 0.12	0.304 ± 0.17
Diarrhoeic calves (n=25)	3.756 ± 0.49	12.886^b ± 2.21	0.423 ± 0.05	0.532 ± 0.07

Mean ± S.E., Different superscripts in columns $P \leq 0.05$.

Table 2(a): Total serum protein, serum albumin, serum globulin and Albumin: Globulin ratio (A: G ratio) in apparently healthy and diarrhoeic cow calves.

Groups	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A : G ratio
Apparently healthy calves (n=6)	6.7^a ± 0.283	3.8^a ± .13	2.9^a ± .3	1.3 ± 0.14
Diarrhoeic calves (n=25)	9.6^b ± 0.48	4.9^b ± 0.28	4.7^b ± 0.24	1.03 ± 0.03

Mean ± S.E., Different superscripts in columns $P \leq 0.05$.

Table 2(b): Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and lactate dehydrogenase in apparently healthy and diarrhoeic cow calves.

Groups	Alanine aminotranferase (IU/L)	Aspartate aminotransferase (IU/L)	Alkaline phosphatase (IU/L)	Lactate dehydogenase (IU/L)
Apparently healthy calves (n=6)	8.7^a ± 0.58	28.6^a ± 1.76	39.5^a ± 1.65	221.6^a ± 15.33
Diarrhoeic calves (n=25)	22.8^b ± 0.98	57^b ± 3.38	69.6^b ± 2.17	286^b ± 14.40

Mean ± S.E., Different superscripts in columns $P \leq 0.05$.

Table 3: Superoxide dismutase (SOD), catalase, glutathione-s-transferase (GST) and glutathione reductase (GR) in apparently healthy and diarrhoeic calves

Groups	SOD (U/mg Hb)	Catalase (µmol/min/mg Hb)	GST (µM/min/g Hb)	GR (µM/min/g Hb)
Apparently healthy calves (n=6)	$9.2^{a} \pm 0.63$	$59^{a} \pm 4.13$	$63.45^{a} \pm 2.11$	$1.75^{a} \pm 0.17$
Diarrhoeic calves (n=25)	$5.36^{b} \pm 0.35$	$73.4^{b} \pm 1.74$	$32.45^{b} \pm 0.99$	$0.97^{b} \pm 0.06$

Mean ± S.E., Different superscripts in columns $P \leq 0.05$.

References

1. Aebi HE. Catalase. In: Bergmeyer, H. O. (Ed.). Methods of Enzymatic Analysis, Vol III. Academic Press, New York, 1983.
2. Alsaad KM, AL-Obaidi QT, Hassan SD. Clinical, haematological and coagulation. Studies of bovine viral diarrhoea in local iraqi calves. Bulgarian J of Vet. Med. 2012; 15(1):44-50.
3. Benjamin MM. Outline of Veterinary Clinical Pathology. 3rd Edn. Reprint, The Iowa State University Press Ames. Iowa, USA, 2013.
4. Botezatu A, Vlagioiu C, Codreanu M, Oraşanu A. Biochemical and haematological profile in cattle effective. Bulletin UASVM Vet. Med. 2014; 71(1):27-30.
5. Brar APS, Ahuja CS, Sood NK, Sandhu BS, Gupta K. Hematological changes in neonatal diarrheic calves of different age groups. Indian J of Vet. Pathol. 2015; 39:75-77.
6. Brooks HW, Gleadhili A, Wagstaff AJ, White DG. Fallibility of plasma urea and creatinine as indices of renal function in diarrhoeic calves treated with conventional or nutritional oral rehydration solutions. Vet. J. 1997; 154:35-39.
7. Carlberg I, Mannervik B. Glutathione reductase. Methods in Enzymology. 1985; 113:484-490.
8. Constable PD. Antimicrobial use in the treatment of calf diarrheoa. J. Vet. Intern. Med. 2004; 18:8-17.
9. Doumas BT, Arends RL, Pinto PVC. Standard Methods of Clinical Chemistry, Academic Press, Chicago. 1972; 7:175-189.
10. Duncan JR, Prasse KW, Mahaffey EA. Veterinary Laboratory Medicine: Clinical Pathology, 3rd edn., The Iowa State University Press, Ames, Iowa, USA, 1994.
11. Fayet JC, Overwater J. Prognosis of diarrheoa in the newborn calf: statistical analysis of blood biochemical data. Ann. Rech. Vet. 1978; 9:55-61.
12. Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-transferase: The first enzymatic step in mercapturic acid formation. J of Biological Chemistry. 1974; 249:7130-7139.
13. Jubb KVF, Keneddy PC, Palmer N. Pathology of domestic animals, 3rd ed. Academic Press Inc., London, 1993.
14. Kaneko JJ. Serum proteins and the disproteinemias. In: Clinical biochemistry of domestic animals, JJ Kaneko, JW. Harvey & ML. Bruss, (Ed.), Academic Press, ISBN 0-12-396305-2, San Diego, California, 1997, pp. 117-138.
15. Malik S, Kumar A, Verma AK, Gupta MK, Sharma SD, Sharma AK *et al*. Haematological profile and blood chemistry of diarrhoeic Calves. J of Ani Health and Product. 2013; 1(1):10-14.
16. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J Biochem. 1974; 47:469-476.
17. Michell AR, Brooks HW, Wagstaff AJ, White DG. The effectiveness of three commercial oral solutions in correcting fluid, electrolyte and acid–base disturbances caused by calf diarrheoa. Br. Vet. J. 1992; 148:507-522.
18. Morgulis S, Beber M, Rabkin I. Studies on the effect of temperature on the catalase reaction: i. effect of different hydrogen peroxide concentrations. J Biol. Chem. 1926; 68:521-533.
19. Pare J, Thurmond MC, Gardner IA, Picanso JP. Effect of birth weight, total protein, serum IgG and packed cell volume on risk of neonatal diarrheoa in calves on two California dairies. Can. J Vet. Res. 1993; 57:241-246.
20. Sharma N, Singh NK, Singh OP, Pandey V, Verma PK. Oxidative stress and antioxidant status during transition period in dairy cows. Asian-Aust. J Anim. Sci. 2011; 24(4):479-484.
21. Singh DD, Kumar M, Choudhary PK, Singh HN. Neonatal calf mortality-An overview. Intas Polyvet. 2009; 10(2):165-169.
22. Singh M, Gupta VK, Mondal DB, Bansal SK, Sharma DK, Shakya M *et al*. A study on alteration in Haemato-biochemical parameters in Colibacillosis affected calves. International J of Adv. Res. 2014; 2(7):746-750.
23. Snedecor GW, Cochran WG. Statistical methods. 8th Ed. Iowa State University Press, Ames, Iowa, USA, 1994.
24. Tietz NW. Text book of Clinical Chemistry. WB. Saunders Co, Philadelphia, 1986b.
25. Tietz NW. Fundamentals of Clinical Chemistry WB Saunders Co., Philadelphia, 1986a.
26. Vegad JL, by Katiyar AK. Cell injury and cell death. In: A Textbook of Veterinary General Pathology. 2nd Ed, CBS Publications, New Dehli, 2008, pp. 32-36.
27. Wroblewski F, La Due JS. Serum glutamic pyruvic transaminase in cardiac and hepatic disease. Proc. Soc. Exper. Biol. Med. 1956; 91:569-571.