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 diarrhoea. 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 (Paratuberculosis), 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 apparently 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 feeding and others animals.

Clinical Complex, LUVAS, Hisar and nearby organized/unorganized farms of Hisar regions aclinical behavior to feeding and others animals. The proposed study was conducted on 31 calves (25 diarrhoeic calves and 6 apparently 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 feeding and others animals.

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

Hematological examination

Haematological studies viz; haemoglobin (Hb), packed cell volume (PCV), total erythrocytic count (TEC), total leucocyte count (TLC), differential leucocytic count (DLC), absolute leucocytic 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) 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), albumin (Doumas et al., 1972), ALT (Wroblewski and La Due 1956), AST (Tietz, 1986a), ALP (Tietz, 1986b) and LDH (Tietz, 1986b) 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). In brief 20 μl of 1% erythrocyte lysate was incubated in 1.0 ml of 30 mM H₂O₂ at 37°C 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 H₂O₂ decomposed/min/mg Hb using 36 as molar extinction coefficient of H₂O₂. The activity of SOD in 1% erythrocyte lysate was determined by the method of Marklund and Marklund (1974). 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). NADPH oxidation was monitored at pH 7.4 and 30°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). 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).

Results and Discussion

The results of haematological studies are presented in Table 1(a-c). It is evident that there was significant (P ≤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 ≤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 malabsorption 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). These findings are in consent with finding of Alsaad et al., 2012; Malik et al., 2013; Botezatu et al., 2014; Singh et al., 2014 and Brat et al., 2015. 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 ≤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 ≤0.05) decrease in albumin: globulin ratio (1.03 ± 0.03) in diarrhoeic calves. A significant increase (P ≤0.05) in activities of ALT (22.8 ± 10.68), AST (57 ± 3.38), ALP (69.6 ± 2.17) and LD (286 ± 14.40) were observed in calves diarrhoea 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 haemoconcentration 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). These finding
In diarrhoeic cow calves, observation of oxidative stress parameters revealed that there were significant (P ≤0.05) decrease in activity of SOD (5.36 ± 0.35), GST (32.45 ± 0.99) and GR (0.97 ± 0.63) whereas as significant (P ≤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/HO₂-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 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 leucocytic count (TLC) in apparently healthy and diarrhoeic calves

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hb (g/dl)</th>
<th>PCV (Per cent)</th>
<th>TEC (10⁶/µl)</th>
<th>TLC (10⁷/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparently healthy calves (n=6)</td>
<td>12.7±0.41</td>
<td>37.7±0.81</td>
<td>8.1±0.49</td>
<td>7.0±0.47</td>
</tr>
<tr>
<td>Diarrhoeic calves (n=25)</td>
<td>9.1±0.54</td>
<td>24.9±1.31</td>
<td>6.2±0.36</td>
<td>17.4±2.42</td>
</tr>
</tbody>
</table>

Mean ± S.E., Different superscripts in columns P ≤ 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

<table>
<thead>
<tr>
<th>Groups</th>
<th>MCH (g/dl)</th>
<th>MCHC (pg)</th>
<th>MCV (fl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparently healthy calves (n=6)</td>
<td>15.8±0.51</td>
<td>33.7±0.43</td>
<td>47±1.78</td>
</tr>
<tr>
<td>Diarrhoeic calves (n=25)</td>
<td>13.6±0.29</td>
<td>32.9±0.71</td>
<td>35.7±1.29</td>
</tr>
</tbody>
</table>

Mean ± S.E., Different superscripts in columns P ≤ 0.05.

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Neutrophil (10⁹/µl)</th>
<th>Lymphocyte (10⁹/µl)</th>
<th>Eosinophil (10⁹/µl)</th>
<th>Monocyte (10⁹/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparently healthy calves (n=6)</td>
<td>2.187±0.14</td>
<td>4.512±0.36</td>
<td>0.262±0.12</td>
<td>0.304±0.17</td>
</tr>
<tr>
<td>Diarrhoeic calves (n=25)</td>
<td>3.756±0.49</td>
<td>12.886±2.21</td>
<td>0.423±0.05</td>
<td>0.532±0.07</td>
</tr>
</tbody>
</table>

Mean ± S.E., Different superscripts in columns P ≤ 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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
<th>A : G ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparently healthy calves (n=6)</td>
<td>6.7±0.283</td>
<td>3.8±0.13</td>
<td>2.9±0.3</td>
<td>1.3±0.14</td>
</tr>
<tr>
<td>Diarrhoeic calves (n=25)</td>
<td>9.6±0.48</td>
<td>4.9±0.28</td>
<td>4.7±0.24</td>
<td>1.03±0.03</td>
</tr>
</tbody>
</table>

Mean ± S.E., Different superscripts in columns P ≤ 0.05.

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Alanine aminotransferase (IU/L)</th>
<th>Aspartate aminotransferase (IU/L)</th>
<th>Alkaline phosphatase (IU/L)</th>
<th>Lactate dehydrogenase (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparently healthy calves (n=6)</td>
<td>8.7±0.58</td>
<td>28.6±1.76</td>
<td>39.5±1.65</td>
<td>221.6±15.33</td>
</tr>
<tr>
<td>Diarrhoeic calves (n=25)</td>
<td>22.8±0.98</td>
<td>57.3±3.38</td>
<td>69.6±2.17</td>
<td>286±14.40</td>
</tr>
</tbody>
</table>

Mean ± S.E., Different superscripts in columns P ≤ 0.05.

~ 12 ~
Table 3: Superoxide dismutase (SOD), catalase, glutathione-s-transferase (GST) and glutathione reductase (GR) in apparently healthy and diarrhoeic calves

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (U/mg Hb)</th>
<th>Catalase (µmol/min/mg Hb)</th>
<th>GST (µM/min/g Hb)</th>
<th>GR (µM/min/g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparently healthy calves (n=6)</td>
<td>9.2 ± 0.63</td>
<td>59 ± 4.13</td>
<td>63.45 ± 2.11</td>
<td>1.75 ± 0.17</td>
</tr>
<tr>
<td>Diarrhoeic calves (n=25)</td>
<td>5.36 ± 0.35</td>
<td>73.4 ± 1.74</td>
<td>32.45 ± 0.99</td>
<td>0.97 ± 0.06</td>
</tr>
</tbody>
</table>

Mean ± S.E., Different superscripts in columns P ≤ 0.05.

References