Molecular investigation of calf diarrhea in relation to changes in some immunological profiles

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ABSTRACT

The aim of this study was to highlight the importance of using PCR to investigate the most important and common pathogens causing calf diarrhea. In addition, to characterize the profiles of some inflammatory cytokines, acute phase proteins, oxidant-antioxidant status, and some immune parameters to clarify their clinical importance during the course of the disease.

The study was conducted on 44 calves, 31 calves were suffering from diarrhea manifestations based on clinical examination (diseased group). The rest 13 calves were apparently healthy and were served as control group. Blood samples were collected from both groups and were assayed for the selected biochemical and immunological parameters. Fecal samples collected directly from the rectum of diarrheic calves were subjected to PCR technique for detecting 5 major bovine enteric pathogens including bovine Coronavirus, bovine Rotavirus, *Salmonella* spp., *E. coli*, and *Cryptosporidium parvum*. The results of PCR indicated that *E. coli*, *Salmonella* and *Cryptosporidium* were the most important pathogens isolated from diarrheic calves at percentages of 38.7 %, 25.8 % and 12.9 % respectively. On the other hand, Rota and Corona viruses were not demonstrated in diarrheic calves. Regarding the selected biochemical and immunological parameters, the results showed that compared to control group, the diseased calves demonstrated significant increases in IL-6, ceruloplasmin, haptoglobin, malondialdehyde and nitric oxide. Significant decreases were observed in total antioxidant capacity, IL-10 and IgA, while no significant changes were reported in serum levels of IgG and IgM. In conclusion, the presented results can document that *E. coli*, *Salmonella* and *Cryptosporidium* are among the most important pathogens resulting in calf diarrhea. The results also provide a confirmation about the importance of proinflammatory cytokines in signaling in the development of calf diarrhea probably through oxidative stress which may rise their values as therapeutic targets for treating diarrhea in calf.

Keywords: PCR, Calf, Diarrhea, Immunity. Oxidative stress.

INTRODUCTION

Calf diarrhea is a multifactorial disease entity that can have serious financial and animal welfare implications in both dairy and beef sucker herds. The disease was found as the most commonly reported disease in young animal and about 75% of early calf mortality in dairy herds has been estimated to be caused by acute diarrhea in the pre-weaning period (Azizzadeh et al., 2012). Until this time, calf diarrhea is still a major cause of productivity and economic loss to cattle producers causing high morbidity and mortality in the cattle industry worldwide (Uhde et al., 2008; Bartels et al., 2010). Economic losses can be attributed to long-term effects on health and...
productivity of surviving calves in addition to treatment and labor costs such as antibiotics, milk replacer and oral rehydration. Infectious diarrhea in calves is mostly associated with enterotoxigenic *Escherichia coli, Cryptosporidium parvum, Coronavirus, Rotavirus*, or some combination of these pathogens (Foster et al., 2009). Among these pathogens, *Escherichia coli* was found as the important cause of diarrhea in neonate. The prevalence of each of pathogen and disease incidence can vary by geographical location of the farms, farm management practices, and herd size (Cho and Yoon, 2014). Different contributing factors that are involved in the occurrence and allow the emergence of the disease include dam, calf immunity, environmental conditions and management practices (Cho and Yoon, 2014).

Because the progression of calf diarrhea can be rapid and since different pathogens or factors may be implicated in the development of the disease, a quick accurate diagnosis is critical not only to confirm the cause but also help producers to implement appropriate treatment protocols. Many laboratory methods for identifying enteric pathogens have been widely used including pathogen isolation and characterization along with histopathology, direct visualization by light or electron microscopy of pathogens in feces or intestinal contents, as well as the detection of antigens by ELISA (Cho and Yoon, 2014). Molecular diagnostics, most often Polymerase Chain Reaction (PCR)-based tests, are becoming more common in diagnostic laboratories for detecting enteric pathogens and present their own challenges in interpretation (Espy et al., 2006). PCR methods are usually more sensitive and may detect lower levels of pathogens that may not be causing disease due to age resistance or those arise from cross-contamination from one calf to another. PCR methods also can detect the dead or stressed bacteria or even in the presence of antibody-antigen complexes (Blanchard, 2012).

This study aimed to highlight the importance of using PCR to investigate the most important and common enteric pathogens causing calf diarrhea. In addition, to characterize the profiles of some inflammatory cytokines, acute phase proteins, oxidant-antioxidant status, and some immune parameters to clarify their clinical importance during the course of the disease.

**MATERIALS AND METHODS**

**Animals**

The present study was conducted on up to 6 months old (7 days till 6 months) 44 bovine calves in different localities in Menufiya governorate, Egypt (in the period from last December 2018 to the first of January 2020). 31 calves were suffering from calf diarrhea. The reset 13 calves were apparently healthy and were considered as control group.

**Samples**

Fecal samples were collected directly from the rectum of diarrheic calves in sterile plastic bags. Fecal samples were subjected to PCR technique for detecting 5 major bovine enteric pathogens including bovine *Coronavirus, bovine Rotavirus, Salmonella spp., E. coli*, and *Cryptosporidium parvum*. Blood samples were collected from both control and diseased groups in plain centrifuge tubes, allowed to clot, centrifuged at 3000 rpm for 15 minutes for separation of serum. Serum samples were stored at -20°C until assayed for the rest biochemical and immunological studies.

**Analytical methods**

**Nucleic acid extraction:**

Total DNA extraction was performed using QIAamp Fast DNA Stool Mini Kit, (Thermoscientific™, Cat No. /ID: 19593, USA) and following the manufacturer's recommendations. RNA extraction was performed using QIAamp Viral RNA Mini Kit (QIAGEN™) according to the manufacturer's instructions.

**Polymerase Chain Reaction (PCR)**

Individual PCR was carried out for each pathogen. Detailed information of the primers used were summarized in (Table 1). DNA samples were tested in 50 µl reaction volumes in a 0.2 ml Eppendorf tube containing 25 µl PCR Master Mix (10X buffer, 10mM d NTPs mixture, Taq polymerase), 2 µl of each primer and 2 µl target DNA, then sterile deionizer water was added to complete the volume to a final of 50 µl, following thermal cycling profile. The thermocycling format was established according to the target pathogen as shown in Table 2. A total of 10 µl of each PCR product was subjected to electrophoresis using a 1.5% ethidium bromide (Sigma-Aldrich, U.S.A.) agarose gel in TBE buffer.
and was visualized under a UV Transilluminator.

**Biochemical and immunological studies:**
Serum level of haptoglobin was determined by ELIZA method using commercial kits of Cell Biolabs, Inc. (USA) according to the method of Sadrzadeh and Bozorgmehr (2004). Serum ceruloplasmin concentration was measured by ELIZA technique using test kits of Beckman Coulter, Inc. (USA) following the method described by Kazmierczak (1996).

Evaluation of oxidative-antioxidant status was done by colorimetric detection of total antioxidant capacity (TAC) according to the method of Koracevic and Koracevic (2001). Serum level of malondialdehyde (MDA) was determined colorimetrically as described by Satoh (1978). Nitric oxide (NO) levels were monitored according to the method of Montgomery and Dymock (1961). All oxidant-antioxidant parameters were evaluated using commercial kits of Biodiagnostics (Egypt). Serum levels of the pro-inflammatory cytokine interleukin-6 (IL-6) and the anti-inflammatory interleukin-10 (IL-10) were detected by ELISA technique using commercial kits supplied by Shanghai Coon Koon Biotech Company (China) and following the manufacturer’s instructions. Concentrations of the immunoglobulins IgA, IgG and IgM in the serum were detected by ELISA using commercial kits supplied by DIMA (Germany) and following the manufacturer's instructions.

**Statistical analysis procedures:**
All the values were presented as mean ± standard deviation (SD). Mean values of diseased group and control group were compared by student’s t test at 0.05 level of probability (Snedecor and Cochran 1980).

**RESULTS**

**Detection of various etiological agents of diarrhea by PCR:**

Individual PCR assays were performed on 44 fecal samples (31 diseased and 13 control) for detecting 5 major bovine enteric pathogens including bovine coronavirus, bovine Rotavirus, *Salmonella* spp., *E. coli*, and *Cryptosporidium parvum*. The prevalence rate of these enteric pathogens isolated from apparently healthy and diarrheic calves by PCR method is shown in Table 3.

The results of PCR indicated that *E. coli* were detected at the most higher prevalence rate whereas the microorganism was isolated from 12 out of 31 fecal samples at a percentage of 38.7% (Fig. 1). *Salmonella* spp. were isolated from 8 out of 31 fecal samples (Fig. 2) while *Cryptosporidium parvum* was isolated from 4 out 31 fecal samples (Fig. 3) at percentages of 25.8% and 12.9% respectively. On the other hand, *Rota* and *Corona* viruses were not demonstrated in diarrheic samples.

**Biochemical and immunological parameters:**
The effect of diarrhea on acute phase response as shown in Table 4 clarified that serum levels of haptoglobin and ceruloplasmin demonstrated a significant (p≤0.05) increase in the diarrhetic calves when compared to the control. Affected calves also showed higher (p≤0.05) serum concentrations of both malondialdehyde and nitric oxide compared to the healthy animals while, serum level of total antioxidant capacity was significantly (p≤0.05) lower in the diseased group compared to the control (Table 4).

Evaluation of some immunologic parameters in the diarrhetic calves cleared that serum value of IL-6 significantly (p≤0.05) increased in affected calves compared to the control but on the other hand, significant (p≤0.05) decrease was observed in the serum level of IL-10 (Table 5). Compared to the control group, the mean serum value of IgA showed a significant (p≤0.05) decrease in the diseased calves, but no significant changes were reported in the serum concentrations of IgG and IgM.
Table 1: Primer sequence, amplicon size and references of the selected diarrhea-induced pathogens.

<table>
<thead>
<tr>
<th>Target agent</th>
<th>Primer name and direction</th>
<th>Nucleotide sequence 5'-3'</th>
<th>Amplicon Size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>F5(F)</td>
<td>TATTATCTTTAGGTGGTATGG</td>
<td>314 bp</td>
<td>Frank et al. (1998)</td>
</tr>
<tr>
<td></td>
<td>F5(R)</td>
<td>GGTATCCTTTAGCAGCAGTATTTTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmonella</td>
<td>invA-F</td>
<td>TTGTITACGCTAATTGACCA</td>
<td>521 bp</td>
<td>Swamy et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>invA-R</td>
<td>CTGACTGTACATCTTGCTGATTTTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>18S rRNA-F</td>
<td>CCACATCTAAAGGAGGCACG</td>
<td>1056 bp</td>
<td>Jellison et al. (2002)</td>
</tr>
<tr>
<td></td>
<td>18S rRNA-R</td>
<td>ATGGATGCATCAGTGTAGCCG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rota virus</td>
<td>VP4-F</td>
<td>TGGTCCTCGCTATTATAAGAGCA</td>
<td>880 bp</td>
<td>Park et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>VP4-R</td>
<td>ATTTCGGACCAATTATAACC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corona virus</td>
<td>BCV-F</td>
<td>GCCGATCAGTCCGACCAATC</td>
<td>407 bp</td>
<td>Tsunemitsu et al. (1999)</td>
</tr>
<tr>
<td></td>
<td>BCV-R</td>
<td>AGAATGTCAAGCGGGGTAT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

E. coli: Escherichia coli.

Table 2: PCR protocol for amplification conditions of PCR products for the detection of the selected diarrhea-induced pathogens.

<table>
<thead>
<tr>
<th>Target agent</th>
<th>Denaturation</th>
<th>Annealing</th>
<th>Extension</th>
<th>Final extension</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temp(°C)</td>
<td>Time</td>
<td>No. of cycles</td>
<td>Temp(°C)</td>
</tr>
<tr>
<td>E. coli</td>
<td>94</td>
<td>30</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Salmonella</td>
<td>93</td>
<td>60</td>
<td>30</td>
<td>42</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>94</td>
<td>60</td>
<td>30</td>
<td>54</td>
</tr>
<tr>
<td>Rota virus</td>
<td>94</td>
<td>60</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>Corona virus</td>
<td>94</td>
<td>60</td>
<td>30</td>
<td>58</td>
</tr>
</tbody>
</table>

E. coli: Escherichia coli; Temp: Temperature.

Table 3: Prevalence rate of different enteric pathogens isolated from apparently healthy and diarrheic calves by PCR method.

<table>
<thead>
<tr>
<th>Fecal isolate</th>
<th>No of +ve samples</th>
<th>Control</th>
<th>Diarrhetic</th>
<th>Total</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>0</td>
<td>12</td>
<td>12</td>
<td>38.7</td>
<td></td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>0</td>
<td>8</td>
<td>8</td>
<td>25.8</td>
<td></td>
</tr>
<tr>
<td>C. parvum</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>12.9</td>
<td></td>
</tr>
</tbody>
</table>

Number of samples =44 (13 control, 31 diseased)

E. coli: Escherichia coli; C. paravum: Cryptosporidium parvum

Table 4: Serum profile of acute phase proteins and Oxidant-antioxidant status of diarrhetic calves compared to the control healthy group. Values are mean ± SD.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (N=13)</td>
</tr>
<tr>
<td>Ceruloplasmin(mg/dl)</td>
<td>21.65±0.35</td>
</tr>
<tr>
<td>Haptoglobin (mg/dl)</td>
<td>109.33±2.52</td>
</tr>
<tr>
<td>TAC (mmol / ml)</td>
<td>3.0±0.62</td>
</tr>
<tr>
<td>Malondialdehyde (nmol/ml)</td>
<td>2.90±0.40</td>
</tr>
<tr>
<td>Nitric oxide (U/ml)</td>
<td>222.67±1.53</td>
</tr>
</tbody>
</table>

TAC: Total antioxidant capacity.
Significant differences in the values between the diseased and the control groups were indicated by (*) at $P \leq 0.05$.

**Table 5**: Interleukins and immunoglobulin profiles of diarrhetic calves compared to the control healthy group. Values are mean ± SD.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (N =13)</th>
<th>Diseased (N =31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL6 (pg/ml)</td>
<td>74±3.40</td>
<td>112.27±4.25*</td>
</tr>
<tr>
<td>IL10 (pg/ml)</td>
<td>72.60±3.90</td>
<td>33.67±3.81*</td>
</tr>
<tr>
<td>IgA (mg/dl)</td>
<td>11.03±1.05</td>
<td>7.50±0.50*</td>
</tr>
<tr>
<td>IgG (mg/dl)</td>
<td>22±5.0</td>
<td>62.67±7.29</td>
</tr>
<tr>
<td>IgM (mg/dl)</td>
<td>30.37±1.52</td>
<td>37.13±4.007</td>
</tr>
</tbody>
</table>

IL6: Interleukin6; IL10: Interleukin10; IgA: Immunoglobulin A; IgG: Immunoglobulin G; IgM: Immunoglobulin M.

Significant differences in the values between the diseased and the control groups were indicated by (*) at $P \leq 0.05$.

**Figure 1**: Molecular diagnosis of *E. coli* by PCR procedure showing positive bands at 314 bp.
- Lane 1: DNA Ladder. Lane 2: control positive. Lane 3: control Negative. Lane 4-15: samples.

**Figure 2**: Molecular diagnosis of *Salmonella spp.* by PCR procedure based on invA gene sequences showing positive bands at 521 bp.
- Lane 1: DNA Ladder. Lane 2: control positive. Lane 3: control Negative. Lane 4-11: samples.
Newborn calves are susceptible to neonatal calf diarrhea (calf scours) especially during their first 28 days of life. Calf diarrhea results in decreases the absorption of essential nutrients from milk and leads to weight loss and dehydratation. Severe cases may be fatal due to dehydration and acidosis that may result in anorexia and ataxia, however, even calves that survive will have poorer performance for the remainder of their lives when compared to healthy calves (Maes et al., 2003 and Berchtold, 2009).

The etiology of calf diarrhea is complex involving both infectious and non-infectious factors (Prescott et al., 2008). Multiple enteric pathogens including viruses, bacteria, and protozoa are involved in the development of the disease (Izzo et al., 2011). Coronavirus, Rotavirus, bacteria (E. coli; Salmonella spp., Clostridium perfringens Type C) and parasites (Cryptosporidium, coccidia) were found to be the most common causes of neonatal calf diarrhea. Among these pathogens, Escherichia coli was found as the important cause of diarrhea in neonate (Cho and Yoon, 2014).

Because of the clinical and economic importance of the disease, laboratory testing is necessary for accurate diagnosis of the problem. Many laboratory methods for identifying enteric pathogens have been widely used including pathogen isolation and characterization, direct visualization by light microscopy or electron microscopy, fecal bacterial culturing, and ELISA (Cho and Yoon, 2014). More recently, detection of antigens by Polymerase Chain Reaction (PCR) has been widely accepted as alternative method for the rapid detection of various bacterial and viral pathogens in clinical specimens from diarrheic calves (Blanchard, 2012).

In the present study, fecal samples of both control and diarrhetic calves were subjected to PCR for detection of 5 major bovine enteric pathogens including bovine Coronavirus, bovine Rotavirus, E. coli, Salmonella spp., and Cryptosporidium parvum. The results showed that E. coli were isolated at the higher prevalence rate at a percentage of 38.7% (12 out of 31 fecal samples) followed by Salmonella spp. and Cryptosporidium parvum at percentages of 25.8% and 12.9% respectively.

In agreement, the incidence of diarrheagenic E. coli in fecal samples of calves collected during November 2015 to March 2016 in Damietta governorate showed the highest incidence rates at percentages of 43% in calves aged 2-7 days, 33% in calves aged 8-14 days followed by 24% in calves aged 15-21 days (Rehab et al., 2016). In the study of Aydin et al., (2001) on diarrheic calves, 92.07% of cases were E. coli positive. In that study, T. vitulorum, Eimeria spp. Cryptosporidium and Salmonella spp. were isolated at percentages of 9.90 %, 5.94 %, 5.94 and %0.99 respectively while 5.94 % of fecal samples revealed neither parasitic nor bacterial infection.

Additionally, Chaurasia, (2001) reported 87 % occurrence of Escherichia coli in the fecal samples of diarrheic calves. These results suggest that Escherichia coli is the most important cause of diarrhea in neonatal calves (Cho and Yoon, 2014).

On the other hand, Seid et al., (2020) found that out of 7 positive samples screened by RT-PCR for molecular characterization, 1 sample was positive for Coronavirus and 4 samples showed Rotavirus. The prevalence rates of enteric pathogen and disease incidence can vary by geographical location of the farms, farm management practices, and herd size (Cho and Yoon, 2014).

Prevention and control of calf diarrhea should be based on a good understanding of the disease pathogenesis and its impact on different body responses and functions. In this respect, the present study aimed to evaluate the effect of calf diarrhea on acute phase response, oxidant-antioxidant status and serum profile of interleukins and immunoglobulins.

Investigation of acute phase proteins in this study revealed a marked increase in serum concentrations of haptoglobin and ceruloplasmin of diarrheic calves as compared to control group. Haptoglobin and ceruloplasmin are acute phase reaction proteins which have been reported to increase.
in animals subjected to external or internal challenges such as infection, inflammation, surgical trauma, or stress (Murata et al., 2004). They are considered integral to the non-specific innate immune response and can be valuable quantitative markers and useful indicators for stress and disease (Petersen et al., 2004).

Previous studies revealed that haptoglobin significantly increased in infected calves with Cryptosporidium, as compared to control group (Al Zubaidi, 2014). Additionally, Hajimohammadi et al., (2013) also found a significant increase in serum ceruloplasmin levels in calves that presented diarrhea, when compared to healthy animals. Also calves that presented diarrhea and tested positive for rotavirus in feces demonstrated higher ceruloplasmin levels in their serum compared to Rotavirus negative animals (Rocha et al., 2016). Similar results of increased serum ceruloplasmin levels were reported by Silva (2011) in Holstein calves experimentally infected with Salmonella Typhimurium and Salmonella Dublin, respectively.

Inflammatory cytokines including IL-6 are known to stimulate the systemic acute phase response as stated by Moshage (1997). This hypothesis was supported by the significant elevation of serum levels of IL-6 seen in the present study.

The results of oxidant-antioxidant status revealed a significant decrease in TAC in diarrheic group compared to control which is consistent with the previous studies (Osman et al., 2020). In this work, serum concentrations of malondialdehyde and nitric oxide were significantly higher in the diarrheic calves compared to their corresponding control values. These findings together with the reduction in TAC provide an evidence of the presence of oxidative stress in the diseased calves.

Biochemically, oxidative stress is associated with increased production of oxidizing species or a significant decrease in the effectiveness of antioxidant defenses with the severity depending on the duration and intensity of such stress.

Malondialdehyde is the major product of lipid peroxidation that reacts with biological structures such as proteins, lipids, carbohydrates, and DNA, causing tissue damage so it reflects the oxidative stress in cells and tissues (Gurer et al., 1998). Similar studies on calf diarrhea proved that serum concentration of MDA and NO increase significantly in Cryptosporidium-infected calves (Kojouri et al., 2012 and Gultekin et al., 2019).

Rachmilewitz et al., (1995) suggested that in acute infectious gastroenteritis, the release of cytokines and inflammatory mediators from activated macrophages is likely responsible for production of NO by phagocytic leukocytes. Sowmyanarayanan et al., (2009) added that it is well known that a large amount of NO is produced over a long period during the inflammatory process of infectious disease. Gultekin et al., (2019) provided another explanation for increased plasma NO concentration in diarrheic calves which occurs due to decreased renal perfusion related to hypovolemia with the resultant decrease in urinary nitrate excretion.

The current findings demonstrated a significant increase in serum levels of the pro-inflammatory IL-6 with a significant decrease in the concentration of the anti-inflammatory IL-10 in the diarrheic calves compared to control. These results are consistent with Stephani et al., (2016) who found greater IL-6 concentrations in the diarrheic calves than in controls.

Further, increased IL-6 gene expression in blood mononuclear cells and in intestinal epithelial cells from scouring calves was observed in calves due to infection with bovine Rotavirus (BRV) (Aich et al., 2007 and Qadis et al., 2014). Pro-inflammatory cytokines were documented to be important in cell signaling that promote inflammatory process and were involved in the upregulation of the inflammatory reactions. They were found also to stimulate the liver to synthesize acute-phase proteins can be justified in three ways, namely its role in the inflammation pathway and inducing the production of acute-phase proteins (Murtaugh et al., 1996).

IL-10 is well known as a cytokine with potent anti-inflammatory properties which counter-regulate production and function of pro-inflammatory cytokines and thus subsiding the inflammatory process. The significant decrease in IL-10 concentrations observed in diarrheic calves compared to the control group suggesting an overwhelming inflammatory reaction and excessive intestinal
damage. Environmental conditions can play a role in the production of bovine IL-10.

Several in vitro studies have shown that high temperature (42°C) and temperature-humidity index (THI) value of > 72 can reduce the proliferation of IL-10-secreting cells compared to low temperature (38.5°C) and a THI value of < 72 (Elvinger et al., 1991 and Lacetera et al., 2005).

With respect to serum immunoglobulins evaluated in this study, the mean values of serum concentrations of IgA were markedly lower in diarrheic group compared to healthy group. On contrary, no significant changes were demonstrated in IgG and IgM between the two groups.

Serum immunoglobulin concentration in dairy cattle is an important indication of immunity against pathogenic microorganism (Quigley, 2002 and Mendonsa, 2011). IgA is known to be the main immunoglobulin found in mucous membranes including those lining the gastrointestinal tract and is an antibody that plays a crucial role in the immune function of mucous membranes.

The obtained findings were nearly similar to those reported by Balikci and AL (2014) who observed significantly lower serum levels of IgG and IgA in calf diarrhea induced by E. coli, Rotavirus and Coronavirus. Youssef et al., (2017) explored the modulation of the gene expression of some immune-inflammatory markers including IgG, and IgA in buffaloes demonstrating digestive disorders and they found downregulation of both IgG and IgA gene expression in blood of all diseased buffaloes compared with controls indicating a state of immunosuppression. Mahmoud et al., (2018) found a highly significant reduction in concentration of IgA in Cryptosporidium–diseased calves which was considered a sign of the suppressive effect of pathogens on the immune system. Further explanation was provided by Athanasiou et al., (2019) who attributed the reduction of serum immunoglobulins in diarrheic calves to the transfer of these immunoglobulins, to the intestinal lumen to counteract the infection.

Moreover, Hummelshoj et al., (2006) have suggested that IL-10 could positively affect the production of IgA and IgG to support the regulation of B and T cell responses. Therefore, the significant reduction in serum levels of IL-10 observed in this study might be a reasonable cause for the decreased IgA levels in diarrheic calves.

In disagreement with the results of IgG and IgM presented in this work, some studies recorded that serum levels of IgG were significantly decreased in diarrheic calves than that of healthy animals (Berge et al., 2009; and Al-Alo et al., 2018). Rocha et al., (2016) found lower IgG concentrations in diarrheic calves with Rotavirus-positive fecal samples while serum IgA did not show significant difference between diarrheic and healthy groups.

Others documented significant decrease in serum IgG concentrations in the febrile diarrheic calves in comparison with that of non-febrile and control animals (Ashgan et al., 2020). The differences between studies can be related to type of pathogen, age of the calf, stage of the disease, strength of passive immunity transfer of immunoglobulins in addition to other environmental conditions.

From the results obtained in this study it can be concluded that E. coli, Salmonella spp. and Cryptosporidium appeared to be the important causes of diarrhea in buffalo calves with the higher prevalence rate was recorded for E. coli. Also, the changes observed in serum concentrations of haptoglobin, ceruloplasmin, IL-6, IL-10 and IgA could provide a good information about the disease process and therefore, rise their clinical importance as valuable diagnostic and prognostic markers for monitoring or treating the disease.

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