ACUTE PHASE RESPONSE IN HOLSTEIN DAIRY CALVES AFFECTED WITH DIARRHOEA

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Summary


The aim of this study was to evaluate serum concentrations of serum amyloid A (SAA), haptoglobin (Hp), interferon-gamma (INFγ) and tumor necrosis factor-alpha (TNF-α) in dairy calves affected with diarrhoea during the first 4 weeks of life, with special reference to etiological agents. To study the acute phase reaction in infectious diarrhoea with E. coli k99, rota and corona viruses and Cryptosporidium parvum, 50 clinically healthy and 50 diarrhoeic calves 1–28 days of age were randomly selected. Blood samples were taken from jugular vein and serum concentrations of serum amyloid A (SAA), haptoglobin (Hp), interferon-gamma (INFγ) and tumor necrosis factor-alpha (TNF-α) were determined. Although all studied acute phase response indicators were significantly increased in diarrhoeic calves, SAA and Hp were two major acute phase proteins that were remarkably increased (p<0.05) compared to healthy animals. According to this study, the SAA and Hp correlated with faecal scores and proved to be reliable indicators of the severity of diarrhea (Spearman rho = 0.22 and 0.23, respectively, P=0.05).

Key words: acute phase response, calf diarrhea, haptoglobin, interferon-gamma, serum amyloid A, tumour necrosis factor-alpha

INTRODUCTION

Acute phase proteins (APPs) are a group of blood proteins whose concentration change in animals subjected to external or internal challenges, such as infection, inflammation, surgical trauma or stress. The hepatic production of APPs increases during the early reaction of the host to infection or tissue damage – the so-called acute phase response (APR) (Baumann & Gauldie, 1994).

The APPs are considered to be non-specific innate immune components involved in the restoration of homeostasis and the restraint of microbial growth before animals develop acquired immunity to a challenge. The circulating concentrations of the APPs are related to the severity of the disorder and the extent of tissue damage in the affected animal; quantification of their concentration can therefore provide diagnostic and prognostic information if proper timing of sampling is assured (Gruys et al., 1994; Eckersall, 2001). During APR, circulating concentrations of APPs change substantially and this makes them good candidates for use in veterinary medicine as quantifiable indicators of inflammation or infection.

Infectious diarrhea remains one of the biggest health challenges in both beef
and dairy industries during the first 4 weeks of life. Calf diarrhoea syndrome has a complex etiopathogenesis and causes important economic losses due to morbidity and mortality, treatment costs, and reduced growth rates in affected calves (Garaicoechea et al., 2006; Reidy et al., 2006). The etiology of this syndrome involves infectious agents (viruses, bacteria, and protozoa) and non-infectious factors such as herd management, host nutritional and immunological condition, which affect the outcome of the disease (Maes et al., 2003; Garaicoechea et al., 2006). Infectious diarrhoea in calves is most commonly associated with different types of enterotoxigenic Escherichia coli, Cryptosporidium parvum, rotavirus, coronavirus, or some combination of these pathogens. Each of these agents leads to diarrhoea through either secretion or malabsorption/maldigestion, though the specific mechanisms and pathways may differ.

Screening for elevated APP values could be useful to identify animals that are, or have recently been, clinically or subclinically diseased (Gånheim et al., 2003). According to the best of our knowledge there is no data available on the level of APPs in diarrhoeic calves or its dependence to etiologic factors.

The aim of this study was to evaluate serum concentrations of serum amyloid A (SAA), haptoglobin (Hp), interferon-gamma (INF-γ) and tumor necrosis factor-α (TNF-α) in dairy calves affected with diarrhoea during the first 4 weeks of life, with special reference to etiological agents such as enterotoxigenic Escherichia coli k99, Cryptosporidium parvum, rotavirus, coronavirus and combination of these pathogens.

MATERIALS AND METHODS

From January 2008 to December 2008, a total of 100 faecal and jugular vein blood samples from diarrhoeic (n=50) and healthy (n=50) calves up to four weeks of age were collected from a 2000-head commercial Holstein dairy herd. After birth, calves were fed 3 kg of dam’s colostrum by nipple bottle and moved to individual pens and fed milk from their dams three times a day for 5 days, after which they were adapted to milk powder. No vaccination against calf diarrhoea was applied.

Visual evaluation of faecal consistency was performed using following criteria: zero or ‘normal’: firm consistency, brown colour, clean and dry perineum and tail; 1: a paste-like consistency, yellow colour and perineum and/or tail smeared with faeces; 2: watery consistency, perineum and/or tail smeared with watery faeces. Faecal scores 1 and 2 were considered as diarrhoea.

Blood samples from the diarrhoeic and non-diarrhoeic calves (control cases, of same age and sex) were taken on the same day, centrifuged (1600×g, 10 min) and sera were frozen at –20 ºC until analyzed for determination of SAA, Hp, INF-γ and TNF-α. SAA was measured by a solid-phase sandwich ELISA (Tridelta Development Plc, Wicklow, Ireland) with analytical sensitivity of 0.3 µg/mL. Hp was measured according to prevention of the peroxidase activity of haemoglobin, which is directly proportional to the amount of Hp. The analytical sensitivity of this test in serum has been determined as 0.0156 mg/mL. Hp was measured using an ELISA kit (AbCys S.A., France).
The faecal samples were kept at \(-20^\circ\text{C}\). A commercial indirect antigen-capture ELISA kit (BGVV B-290, Bio-X Diagnostics, Belgium) employing specific monoclonal antibodies was used to detect \(E. \text{coli k99}\), rota- and corona viruses and \(\text{Cryptosporidium parvum}\) in faecal samples. The ELISA procedure was performed according to the manufacturer's instructions.

Mean concentrations of different acute phase proteins of healthy and diarrhoeic calves in different groups were compared by one-way ANOVA and Tukey's test. Mean concentrations of different acute phase proteins of healthy and diarrhoeic calves in ETEC and coronavirus groups were compared by Kruskal-Wallis test and Mann-Whitney U Test. Spearman rho correlation coefficient was used to report the correlation coefficients between individual parameters and faecal scores. A P value of less than 0.05 was considered significant.

RESULTS

Average faecal scores based on etiological agents were shown in Table 1. They ranged between 1.14 for calves with rotaviral infection and 2.00 in calves affected by ETEC. The cause of diarrhoea and the number of calves affected by each agent or combinations of agents are presented in Table 2. Serum levels of SAA, Hp, interferon-\(\gamma\) and TNF-\(\alpha\) in diarrhoeic calves were significantly higher than those in healthy ones (\(p<0.05\)). Furthermore, it was shown that the levels of SAA and Hp were significantly different in diarrhoeic calves with regard to etiologies (except between coronavirus and rotavirus affected calves).

There was no difference between the levels of SAA and Hp in rotavirus+\(\text{Cryptosporidium}\) and coronavirus+\(\text{Cryptosporidium}\) co-infections. It was also shown that SAA and Hp concentrations were the highest in diarrhoeic calves affected with ETEC k99 (\(P<0.05\)). According to the results of this study, serum concentrations of SAA and Hp were higher in concurrent infections (rotavirus + \(\text{Cryptosporidium}\) and coronavirus + \(\text{Cryptosporidium}\)), when compared to infections caused by rotavirus or coronavirus alone (\(P<0.05\)). There were no significant differences between the levels of INF-\(\gamma\) and TNF-\(\alpha\) among diarrhoeic calves with different etiologies.

Spearman rho correlation coefficients between SAA and Hp with faecal scores were 0.22 and 0.23 (\(P=0.05\)) respectively and those between INF-\(\gamma\) and TNF-\(\alpha\) with faecal scores, 0.04 and \(-0.173\) (\(P=0.1\)), respectively.

<table>
<thead>
<tr>
<th>Etiology of diarrhoea</th>
<th>Average faecal score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rota</td>
<td>1.14</td>
</tr>
<tr>
<td>Corona</td>
<td>1.33</td>
</tr>
<tr>
<td>Crypto</td>
<td>1.16</td>
</tr>
<tr>
<td>ETEC</td>
<td>2.00</td>
</tr>
<tr>
<td>Rota+Crypto</td>
<td>1.80</td>
</tr>
<tr>
<td>Corona+Crypto</td>
<td>1.66</td>
</tr>
<tr>
<td>None</td>
<td>1.17</td>
</tr>
</tbody>
</table>

Table 1. Average faecal scores based on etiological agents in diarrhoeic groups: Rotavirus (Rota), Coronavirus (Corona), Cryptosporidium parvum (Crypto), enterotoxigenic Escherichia coli (ETEC), Rotavirus+Cryptosporidium (Rota+Crypto), Coronavirus+Cryptosporidium (Corona+Crypto) and diarrhoea of non-specified etiology (none)
Table 2. Mean±SEM serum concentrations of serum amyloid A (SAA), haptoglobin (Hp), tumor necrosis factor- α (TNF-α) and interferon-gamma (INF-γ) in healthy and diarrhoeic calves affected with Rotavirus (Rota), Coronavirus (Corona), Cryptosporidium parvum (Crypto), enterotoxigenic Escherichia coli (ETEC), Rotavirus+Cryptosporidium (Rota+Crypto), Coronavirus+Cryptosporidium (Corona+Crypto) and diarrhoea of non-specified etiology (None)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy (n=50)</th>
<th>Rota (n=7)</th>
<th>Corona (n=3)</th>
<th>Crypto (n=6)</th>
<th>ETEC (n=3)</th>
<th>Rota+Crypto (n=5)</th>
<th>Corona+Crypto (n=3)</th>
<th>None (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAA (µg/mL)</td>
<td>5.18±0.38</td>
<td>26.31±0.68</td>
<td>26.34±0.26</td>
<td>19.82±0.95</td>
<td>93.03±1.52</td>
<td>43.08±1.06</td>
<td>41.72±0.73</td>
<td>13.78±0.54</td>
</tr>
<tr>
<td>Hp (g/L)</td>
<td>0.086±0.003</td>
<td>0.33±0.01</td>
<td>0.34±0.008</td>
<td>0.25±0.005</td>
<td>0.73±0.02</td>
<td>0.49±0.01</td>
<td>0.486±0.008</td>
<td>0.18±0.004</td>
</tr>
<tr>
<td>INF-γ pg/dL</td>
<td>14.36±0.75</td>
<td>46.92±0.9</td>
<td>47.89±0.19</td>
<td>44.75±0.98</td>
<td>46.88±0.94</td>
<td>46.81±1.49</td>
<td>47.41±2.05</td>
<td>45.18±0.57</td>
</tr>
<tr>
<td>TNF-α pg/dL</td>
<td>25.32±18.00</td>
<td>55.01±1.18</td>
<td>56.17±2.51</td>
<td>53.75±0.97</td>
<td>55.3±1.22</td>
<td>55.36±0.8</td>
<td>54.58±2.05</td>
<td>56.25±0.55</td>
</tr>
</tbody>
</table>

* P<0.05 between healthy and diarrhoeic groups for each acute phase response indicator; different letters within a row show significant differences in diarrhoeic calves with regard to etiologies (P<0.05).
DISCUSSION

In bovines, two major serum APPs proteins have been recognized: serum amyloid A (SAA) (Horadagoda et al., 1993) and haptoglobin (Hp) (Eckersall & Conner, 1988). They have been used to evaluate inflammatory conditions in clinical or experimental cattle studies (Alsemgeest et al., 1994; Heegaard et al., 2000; Eckersall et al., 2001). Acute phase proteins (APPs) are mostly glycoproteins produced in lower (negative APP) or increased (positive APP) amounts in response to inflammatory processes. Previous studies have shown that haptoglobin is an important positive APP in cattle. Under normal conditions, Hp is absent or present in very low concentrations in serum, ranging from 0.05 to 0.10 g/L, but the concentration may increase 50–100 times in response to different bacterial, viral, parasitic and inflammatory diseases (Conner et al., 1989; Skinner et al., 1991; Alsemgeest et al., 1994; Godson et al., 1996; Hirvonen et al., 1996; Heegaard et al., 2000; Ganheim et al., 2003; Murata et al., 2004).

Horadagoda et al. (1999) reported that SAA and Hp in animals were raised in acute rather than chronic cases. Hp assessment was used to identify the effectiveness of antibiotic treatment in feedlot cattle (Wittum et al., 1996) and in cattle suffering from toxic puerperal metritis (Smith et al., 1998). Monitoring of serum Hp concentration has also been used to assess the host response to experimentally induced mastitis (Hirvonen et al., 1999), the response to a prolonged low dose of lipopolysaccharide (Werling et al., 1996) and in an antigen-induced model of arthritis in sheep (Highton et al., 1997).

According to our study, SAA and Hp increased significantly in calves with diarrhea. The increase was more pronounced in ETEC and mixed rotavirus+Cryptosporidium and coronavirus+Cryptosporidium infections. The acute phase response is stimulated by the release of cytokines such as interleukin-1, interleukin-6 and tumour necrosis factor α (TNF-α) from macrophages and monocytes at the site of inflammatory lesions or infection (Dinarello et al., 1984; Beutler et al., 1986; Heinrich et al., 1990).

A more objective parameter could be of significant benefit in determining the onset of infection and monitoring its course. It has been reported that ETEC can produce secretory diarrhoea via two virulence factors, K99 fimbria and heat stable toxin in the first 4 days of life and rarely leads to diarrhoea in older calves or adult cattle. According to previous studies, ETEC K88 that cause infection of intestinal cells can induce an inflammation-associated response in pigs (Roselli et al., 2003; 2006). Enteric ETEC infections may also result in secondary septicaemia accompanied by severe diarrhoea and dehydration (Fairbrother & Ngeleka, 1994). In such cases, ETEC may pass through the intestinal mucosa, probably by endocytic uptake into intestinal epithelial cells or through the intercellular spaces between epithelial cells, to locate in the mesenteric lymph nodes before entering the bloodstream, resulting in a generalized infection with bacterial dissemination in extraintestinal organs (Gyles et al., 2004). Although the mechanism of ETEC K99 inflammation has not been yet reported, according to our findings ETEC can induce SAA and Hp by rapid release of large amounts of LPS stimulating the overproduction of inflammatory mediators including TNF-α, interleukin-1 and interleukin-6 (Qureshi et al., 1991; Whitfield et al., 1994).
Rotaviruses preferentially target the mature villous enterocytes and spare the crypts, generally causing moderate villous damage. Malabsorption will then occur because of the loss of surface area, and unabsorbed glucose and other carbohydrates create an osmotic load pulling fluid into the lumen. Furthermore, fluid secretion from the crypts increases the amount of fluid in the intestinal lumen relative to villous absorption, which leads to diarrhoea (Argenzio, 1985; Torresy Medina et al., 1985; Ramig, 2004). However, the severity of clinical signs does not always correlate with histologic damage to the villi. The pathophysiology of coronaviral diarrhoea in calves is highly similar to overlaps significantly with that caused by rotavirus. Cryptosporidiosis, similar to rotavirus and coronavirus infections, causes destruction of intestinal epithelia resulting in a reduction of enzymatic activity and a decrease in the absorptive surface, finally leading to malabsorption and malabsorption followed by diarrhoea (Foster & Smith, 2009).

Concurrent infections of C. parvum with rotavirus and coronavirus have been described (Naciri et al., 1993). Bartels et al. (2010) found concurrent infections of C. parvum with rotavirus in 7.8% of diarrhoeic calves, and increased mortality due to concurrent infections of C. parvum with other enteric pathogens has been reported (Naciri et al., 1993). Concurrent infection of Cryptosporidium spp. with rotavirus and coronavirus deteriorates the malabsorption in comparison to single infection (Foster & Smith, 2009), and can lead to more severe illness. Some authors believe that the presence of concurrent infections of Cryptosporidium spp. and other causes of diarrhoea affects the severity of diarrhoea (O’Handley et al., 1999). Furthermore, it was also found that the levels of INF-γ and TNF-α are not the reliable indicators of the score (severity) of diarrhoea (Spearman rho = 0.04 and –0.173, respectively, P=0.1).

Finally, according to this study, it could be concluded that SAA and Hp are two major acute phase proteins that were increased remarkably in diarrhoeic calves up to four weeks of age and that could be used as reliable indicators of diarrhoea’s severity.

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Acute phase response in Holstein dairy calves affected with diarrhea

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