Some serum acute phase proteins and immunoglobulins concentrations in calves with rotavirus, coronavirus, *E. coli* F5 and *Eimeria* species

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Summary

The purpose of this study was to evaluate the changes in the serum concentrations of haptoglobin (Hp), serum amyloid A (SAA) and *IgG*, *IgA* in calves with diarrhea caused by rotavirus, coronavirus, *Escherichia coli* F5 and *Eimeria* species. The experiment was carried out on 40 diarrhoeic and 10 non-diarrhoeic calves (group C). A total of 13 calves were infected with rotavirus or coronavirus (group V), 12 calves with *E. coli* F5 (group B) and 15 calves with *Eimeria* species (group P). SAA and Hp levels of calves in groups V, B and P were statistically higher than group C (P<0.05). SAA and Hp levels of the group B and group P were significantly higher than the group V (P<0.05). SAA and Hp levels in group B were not significantly higher than the group P. The levels of *IgG* and *IgA* were found to be lower in groups B and V compared to other groups. There was a negative correlation between immunoglobulins and the levels of serum Hp and SAA in groups B and V (r=-0.315 and r=-0.369, respectively, P<0.05). Serum SAA, Hp, *IgA* and *IgG* levels could be useful for the diagnosis and differential diagnosis of diarrhea caused by rotavirus, coronavirus, *E. coli* F5 and *Eimeria* species.

Key words: Diarrhea, Serum amyloid A, Haptoglobin, *IgA*, Calves

Introduction

Calves are at greatest risk of developing diarrhea during the first month of life, and the risk then decreases with age (De La Fuente *et al*., 1998; Garcia *et al*., 2000). Substantial economic loss occurs as a result of increased morbidity and mortality, treatment costs and reducing growth rates. The diarrhoeal syndrome has a complex aetiopathogenesis because various infectious agents, either alone or in combination, may be associated with field outbreaks. In addition, environmental, nutritional and management factors influence the severity and outcome of the disease. Rotavirus, bovine coronavirus, *Escherichia coli* F5, and *Cryptosporidium* species are internationally recognized as the most important enteropathogens in acute diarrhea in young calves (Reynolds *et al*., 1986; De La Fuente *et al*., 1998). Among the protozoa, *Eimeria* are considered relevant causes of diarrhea in calves beginning at approximately 3 weeks of age (Svensson, 1993).

Acute phase proteins (APPs) are a group of plasmatic proteins whose concentration changes after infection, inflammation or surgical trauma, factors that trigger the acute phase response (Murata *et al*., 2004; Petersen *et al*., 2004). Serum concentration of positive APPs, such as haptoglobin (Hp), α₁ acid glycoprotein (AGP), C-reactive protein (CRP) and serum amyloid A (SAA) increase during acute phase response (Murata *et al*., 2004). APP levels are not suitable for establishing a specific diagnosis, but quantification of their concentration can provide objective information about the extent of ongoing lesions in individual animals (Eckersall, 2000). APPs may provide alternative means of monitoring animal health. An increased focus on the application of APPs for this purpose has been developed (Skinner *et al*., 1991). APPs may be useful for providing information about the stage of clinical and subclinical infections and also for prognosis of their severity. Hp and SAA are important bovine APPs which increase in serum, for example, during viral and bacterial diseases (Murata *et al*., 2004) but are absent or present in very low levels in healthy animals (Ganheim *et al*., 2003). There are relatively few reports of APP induction by parasite infections although infections with *Babesia canis*, *Anaplasma marginale*, and Coccidiosis might be accompanied by increased levels of APPs (Shapiro *et al*., 1992; Lobetti *et al*., 2000; Hashemnia *et al*., 2011). Studies show that SAA and Hp have been found useful in herd screenings to identify cows with inflammatory diseases (Saini *et al*., 1998; Karreman *et al*., 2000). However, Sorensen *et al*. (2006) recommended coupling the determination of the concentrations of two or three APPs for improving the infection/inflammation diagnosis.

A few documents (Pourjafar *et al*., 2011; Hajimohammadi *et al*., 2013) describe the changes in serum concentrations of APPs in calves with diarrhea. Moreover, most of these studies only assessed diarrhea as background for APPs investigations of other illnesses such as respiratory diseases. So, the purpose of this study was to evaluate the changes in the serum concentrations of *IgG*, *IgA*, Hp and SAA in calves with diarrhea caused

by rotavirus, coronavirus, *E. coli* F5 and *Eimeria* species.

Materials and Methods

Study area and animals

The study was conducted in the province of Elazığ in Turkey, during 2011-2012. Forty diarrhoeic (aged between 2 and 48 days) and 10 non-diarrhoeic calves (aged between 10 and 30 days) (group C) were used in the study.

A total of 13 calves were infected with virus (9 with rotavirus and 4 with coronavirus) (group V), 12 calves with *E. coli* F5 (group B) and 15 calves with *Eimeria* species (group P). Faeces samples were taken from the rectum of all diarrhoeic and non-diarrhoeic calves.

Diagnosis of the causes of diarrhea

Detection of rotavirus, coronavirus and *E. coli* F5 in stool samples was done with commercial *in-vitro* Rapid Diagnostic Test (Quatro Vet Uni-Strip Kit, C-1540, CorisBioConcept, Belgium). The stool samples were centrifuged with saturated NaCl solution for flotation and were examined under the light microscope (Soulsby, 1982). After flotation examination of stools, the detected oocysts were sporulated in 2.5% potassium dichromate at 22°C and the species identification was performed.

Sample collection and biochemical assays

Blood samples were taken by veni puncture of the jugular vein into silicone vacutainer tubes. The blood samples were centrifuged at 1500 g for 10 min at 4°C. Serum were carefully separated from the packed cells and stored at -20°C until measurement of serum Hp and SAA concentrations.

Serum Hp levels were measured based on prevention of the peroxidase activity of hemoglobin, 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 for Hp by the manufacturer (Tridelta Development Plc., Wicklow, Ireland).

Serum SAA levels were measured by a solid phase sandwich-ELISA. The analytical sensitivity of this test in serum has been determined as 0.3 μg/ml for SAA by the manufacturer (Tridelta Development Plc., Wicklow, Ireland).

Serum *IgA* and *IgG* concentrations were determined using Single Radial Immunodiffusion (SRID) commercial test kit (RID Kits, WMRD, Pullman, Wash., USA).

Statistical analysis

The data were analyzed by one-way ANOVA using SPSS/PC software. Duncan's multiple range test was used to detect significant differences between means. All values were expressed as mean and standard error (SEM); $P<0.05$ was accepted as statistically significant.

Results

The diarrhoeic calves had fever, anorexia, general weakness, loss of condition, moderate and severe profuse watery diarrhoea and dehydration. Non-diarrhoeic calves were examined and judged to be clinically normal. The calves used in this study were within 1-2 days at the first appearance of clinical symptoms. Calves with coccidiosis had bloody diarrhea.

Table 1 shows number of positive results on the SAA and Hp levels of group V, group B, group P and group C.

Table 2 shows the data comparing the SAA and Hp levels of groups V, B, P and C. The increases at Hp levels in the groups B, P and V were (0.896 ± 0.102 mg/ml), (0.762 ± 0.083 mg/ml) and at least a group V (0.412 ± 0.095 mg/ml), respectively. The increases at SAA levels were 46.27 ± 9.65 μg/ml, 87.24 ± 8.86 μg/ml and 103.54 ± 12.84 μg/ml in V, P and B groups, respectively. Mean SAA and Hp levels of calves in groups V, B and P were statistically higher than group C. However, mean SAA and Hp levels of the group B and group P were significantly higher than the group V ($P<0.05$). Mean SAA and Hp of the group B were not significantly higher than the group P ($P>0.05$). Serum *IgG* and *IgA* levels in calves which were detected *E. coli* F5 (group B), rotavirus and coronavirus (group V) were also found to be low. There was a negative correlation between B and V groups for immunoglobulins and the levels of serum Hp and SAA (r=-0.315 and r=-0.369, respectively, $P<0.05$). Serum *IgG* and *IgA* levels in groups P and C were close to normal limits.

Discussion

In calves, diarrhea is considered as one of the most

Table 1: Numbers and ratios of calves according to SAA (>14 μg/ml) and Hp (>0.1 mg/ml) values

Parameters	Group C	Group V	Group B	Group P
SAA >14 μg/ml	0/10 (0%)	10/13 (76.9%)	7/7 (100%)	9/10 (90%)
Hp >0.1 mg/ml	0/10 (0%)	11/13 (84.6%)	7/7 (100%)	9/10 (90%)

Table 2: Mean ± SE of Hp, SAA, *IgG* and *IgA* levels in calves with diarrhea (group V, B, P) and in healthy calves (group C)

Parameters	Group C	Group V	Group B	Group P
SAA (μg/ml)	11.63 ± 1.15[a]	46.27 ± 9.65[b]	103.54 ± 12.84[c]	87.24 ± 8.86[c]
Hp (mg/ml)	0.073 ± 0.010[a]	0.412 ± 0.095[b]	0.896 ± 0.102[c]	0.762 ± 0.083[c]
IgG (mg/dl)	1650.2 ± 178.3[a]	624.6 ± 154.2[b]	553.5 ± 184.8[b]	1476.6 ± 256.7[a]
IgA (mg/dl)	385.2 ± 26.4[a]	280.1 ± 34.6[b]	268.4 ± 40.3[b]	368.9 ± 24.5[a]

Different superscript letters ([a, b, c]) within same row indicate significant ($P<0.05$) differences among groups

important diseases, because the resulting economic loss following mortality, treatment costs, and decrease of growth rate would be detrimental (Maes *et al.*, 2003). In the study, rota and coronavirus were identified as viral group, *E. coli* as bacterial group and *Eimeria* as protozoal group by looking at the values of Hp and SAA to execute the changes in APPs in calves with diarrhoea. Pourjafar *et al.* (2011) reported that the SAA and Hp correlated with faecal scores and proved to be reliable indicators of the severity of diarrhea. Also, Hajimohammadi *et al.* (2013) indicated that monitoring the APP responses in diarrheic calves with different clinical signs could be useful as prognostic tools and facilitate treatment decisions. However, studies on acute phase reactions in field conditions are more complicated, because the timing of initial infection is often unknown, and it is difficult to determine the phase of infection.

Hp is a major APP in ruminants, in which species it has a slight circulating level in normal animals, but increases over 100 fold on stimulation. In healthy cattle the serum Hp concentration is <20 mg/L but can increase to >2 g/L within 2 days of infection (Eckersall and Bell, 2010). In this study, the calves were recruited within 2 day after the start of clinical symptoms in patients. Petersen *et al.* (2004) reported that clinical signs of lameness, diarrhoea, respiratory disease, and ear necrosis were reflected in a high serum Hp concentration. Many studies have reported the serum Hp as a clinically useful parameter for measuring the occurrence and severity of inflammatory responses in cattle with enteritis, peritonitis, endometritis, mastitis, pneumonia, endo-carditis, abscesses, and other natural or experimental infectious conditions (Horadagoda *et al.*, 1994; Godson *et al.*, 1996; Nazifi *et al.*, 2011; Balikci *et al.*, 2013). The difference between the groups was most obvious for Hp, indicating that this may be the most useful predictive APPs and is in agreement with Carter *et al.* (2002) who concluded that analysis of serum Hp was a better tool for discrimination between calves that became ill and those that did not, compared to other APPs. Ganheim *et al.* (2007), reported healthy calves were used in determining serum Hp levels 0.06 to 1.23 g/L. Skiner *et al.* (1991) and Saini *et al.* (1998), serum Hp concentration from 0.2 to 0.4 g/L between the mild, 1-2 g/L was defined as a level of severe infection. In this study, serum Hp levels increased 50-100 times in all diarrhoeic groups. The increases at Hp levels in the groups B, P and V were 0.896 ± 0.102 mg/ml, 0.762 ± 0.083 mg/ml and 0.412 ± 0.095 mg/ml, respectively. The Hp levels of control group (0.073 ± 0.010 mg/ml) were in normal levels as previously reported for the calves (Ganheim *et al.*, 2007). Hp levels were statistically higher (P<0.05) in the groups B, P and V when compared with group C. However, it was observed that the mean concentrations of Hp were higher in groups B and P than in group V (P<0.05). Our findings on the systemic Hp response are in agreement with other reports (Godson *et al.*, 1996; Petersen *et al.*, 2004; Pourjafar *et al.*, 2011; Balikci *et al.*, 2013; Hajimohammadi *et al.*, 2013). Approximately a hundred times of statistically higher levels of Hp in diarrhoeic

groups compared to group C can be considered as an indication of the acute and severe infection. However, the lowest increase in group V can be attributed to the resistance of patients or prolonged infection period. In this study, the acute phase response induced by diarrhea diseases was generally the same or higher than previously reported for bacterial infections in calves. However, Pourjafar *et al.* (2011) reported that the levels of SAA and Hp were significantly different in diarrhoeic calves with regard to etiologies and higher SAA and Hp concentrations in diarrhoeic calves affected with ETEC K99. Serum amyloid A responded more rapidly to infection, but Hp concentrations correlated better with diseasese verity (fever, anorexia, general weakness, loss of condition, moderate and severe profuse watery diarrhoea and dehydration). Hajimohammadi *et al.* (2013) found that serum Hp and SAA concentrations in diarrheic calves and in calves that had severe clinical signs were 270 mg/L, 27.77 mg/L and 471 mg/L, 56.34 mg/L, respectively.

SAA is a remarkably moderate acute phase protein in cattle increasing around 2-5 times during an acute phase response (Alsemgeest *et al.*, 1994; Gruys *et al.*, 1994). Because of the difficulty of measuring serum SAA levels, the application of SAA assays to veterinary diagnosis has not been as widespread as that of Hp assays. SAA has been suggested to be more useful in distinguishing acute and chronic inflammations than neutrophil counts and white blood cell counts (Horadagoda *et al.*, 1999). Howover, SAA is elevated more by acute rather than chronic inflammatory conditions (Horadagoda *et al.*, 1999). This protein is also increased following experimental infection with *Mannheimia haemolytica*, bovine respiratory syncytial virus, Border disease virus and in experimentally induced and naturally-occurring cases of mastitis (Horadagoda *et al.*, 1994; Heegaard *et al.*, 2000; Eckersall *et al.*, 2001; Balikci *et al.*, 2013). Serum SAA is reported to be more sensitive to stimulation (Horadagoda *et al.*, 1999; Heegaard *et al.*, 2000), and as an increase can also be induced by other factors than disease, such as stress (Alsemgeest *et al.*, 1995) it may be less suitable as an indicator of health problems. Serum SAA levels in healthy calves averaging 14 mg/L have been reported (Lomborg *et al.*, 2008). In this study, SAA levels increased 4-10 times in all patient groups. The increases at SAA levels were 46.27 ± 9.65 µg/ml, 87.24 ± 8.86 µg/ml and 103.54 ± 12.84 µg/ml in V, P and B groups, respectively. The SAA levels of group C (11.63 ± 1.15 µg/ml) were in normal levels as previously reported for the calves (Lomborg *et al.*, 2008). Serum SAA levels were statistically higher (P<0.05) in the groups B, P and V when compared with group C. However, mean SAA levels of the group B and group P were significantly higher than group V (P<0.05). These observations are in accordance with previous results (Alsemgeest *et al.*, 1994; Alsemgeest *et al.*, 1995; Horadagoda *et al.*, 1999; Orro *et al.*, 2011; Pourjafar *et al.*, 2011; Hajimohammadi *et al.*, 2013). Four-ten times of statistically higher levels of SAA in diarrhoeic groups

compared to group C can be considered as an indication of APR. However, the lowest increase in group V can be attributed to the short-term presence of SAA in blood.

Calves are at risk of an inflammatory process from the first few hours of life as the increase in Hp concentrations after infection with the bacterial agent occurs within 24 h (Ganheim *et al.*, 2003). The highest Hp levels, as well as the highest individual differences, occurred in the calves of the group showing failure of passive transfer (Ig levels below 5 g/L at the 48th h of life). The Hp and SAA levels were normal in newborn calves. Diseases that appeared before the fourth day of life induced an increase in SAA levels in all diseased calves, but Hp levels were only raised in two out of eight individuals. The authors indicated that because the SAA concentration at birth is very low and increases rapidly in diseased calves, it might be used as marker of infection during the perinatal period (Alsemgeest *et al.*, 1995). There was failure of passive transfer (serum *IgG* level below 5 g/L) in two calves and were from the same farm in which only 31% of the calves achieved serum *IgG* levels >10 g/L (Stefaniak *et al.*, 2003). In our previous study, the relationship between neonatal diarrhoeic and failure of passive transfer in neonatal calves had been explained (Al and Balikci, 2012). In this study, the levels of *IgG* and *IgA* in calves which detected *E. coli* F5 (group B), rotavirus and coronavirus (group V) were also found to be low. There was a negative correlation between B and V groups for immunoglobulins and the levels of serum Hp and SAA (r=-0.315 and r=-0.369, respectively, P<0.05). There were two probable reasons for the rise in APP levels: either the inflammation occurred very early (possibly before birth), which might have caused the poor transfer of colostrum Ig, or a poor immune protection enabled early disease appearance. The results showed that in calves with diseases or in calves with low levels of colostrum protection, the probability of a rise in APP levels occurred more frequently. It was concluded that the calves had been at risk of an inflammatory process from the first few hours of life, since the increase in Hp concentrations after infection with the bacterial agent occurs within 24 h. Also, serum *IgG* and *IgA* levels in groups P and C were close to normal limits.

It can be concluded from the results obtained in this study that, a blood sample would provide information that could be useful for the diagnosis and differential diagnosis of viral, bacterial and protozoal diarrhea based on the serum Hp, SAA, *IgA* and *IgG* values. In addition, watching the APP concentrations during the infection may be useful for monitoring the prognosis or treatment of diarrhoeic diseases in calves.

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