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EVALUATION OF SERUM HAPTOGLOBIN IN CLINICALLY HEALTHY CATTLE AND CATTLE WITH INFLAMMATORY DISEASES IN SHIRAZ, A TROPICAL AREA IN SOUTHERN IRAN

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Summary

Nazifi, S., A. Rezakhani, M. Koohimoghadam, M. Ansari-Lari & Z. Esmailnezhad, 2008. Evaluation of serum haptoglobin in clinically healthy cattle and cattle with inflammatory diseases in Shiraz, a tropical area in southern Iran. *Bulg. J. Vet. Med.*, **11**, No 2, 95–101.

This study was designed to determine a reference range of haptoglobin (Hp) in Iranian Holstein cattle of different ages and of both sexes, and to evaluate Hp as marker in some inflammatory diseases in Shiraz, a tropical area in southern Iran. Blood samples were collected from the coccygeal vein of 50 clinically healthy cattle and 36 cows with various inflammatory diseases (acute diffuse traumatic reticuloperitonitis, digestive tract infection and abomasal displacement, anaplasmosis, theileriosis, acute respiratory infections and clinical mastitis). Hp was measured according to preservation of the peroxidase activity of haemoglobin which is directly proportional to the amount of haptoglobin. There was no significant difference in Hp concentrations between healthy Holstein cattle from the studied different age groups as well as between both sexes. Pregnancy and lactation had no significant effects on blood Hp concentrations. Statistically significant differences in Hp were observed between clinically healthy and diseased cows ($P<0.05$). The results of the present study revealed that serum Hp was a sensitive factor in various inflammatory conditions in cattle.

Key words: clinically healthy cattle, haptoglobin, inflammatory diseases, Iran

INTRODUCTION

Haptoglobin (Hp) is an α_2-globulin, synthesized in the liver. It is one of acute phase proteins whose serum levels increase in acute infections (Burtis & Ashwood, 1999). It is believed that in cattle, Hp is involved in the regulation of lipid metabolism (Nakagawa *et al*., 1997) and acts as immunomodulator (Murata & Miyamoto, 1993). The reference value of serum Hp was determined in cattle (Lipperheide *et al.*, 1997; Nakagawa *et al*., 1997). In apparently healthy animals (mixed breed and age), serum Hp reference value was <0.35 g/L (Horadagoda *et al*., 1994).

Skinner *et al*. (1991) evaluated serum Hp in inflammations and introduced Hp as an inflammatory indicator in cattle. Determinations and evaluations of serum Hp showed that this protein could be valuable in the diagnosis of infection (Alsemgeest *et al*., 1994). Hirvonen *et al*. (1996) reported increased Hp concentrations in heifers with mastitis. According to Gron-

lund *et al*. (2003), Hp increased rapidly in milk and serum in the acute phase of staphylococcal mastitis in cows. The stage of disease can be better evaluated by monitoring more than one acute phase protein, so that both chronic and acute conditions should be evaluated and characterised by acute phase protein profiling (Eckersall, 2004). Measurement of Hp could be a useful tool for evaluation of health in calf herds (Ganheim *et al*., 2007). There were no published reports about the concentration of Hp in some inflammatory diseases (e.g. theileriosis due to *Theileria annulata* and anaplasmosis due to *Anaplasma marginale*). Therefore, the present study was conducted to determine a reference range for Hp in cattle of different ages from both sexes and to evaluate Hp as an inflammatory marker in various inflammatory diseases in Shiraz, a tropical area in southern Iran.

MATERIALS AND METHODS

Fifty clinically healthy Holstein cattle from a dairy farm in the Fars province, Iran, were selected for this study. They were of both sexes in 3 age groups (<2, 2–4 and >4 years). Calves under the age of 3 months were fed milk and then concentrates. Barley, corn and concentrates were used in the diet of dairy cows. Dry cows were fed barley and corn. All cattle were vaccinated against foot and mouth disease, brucellosis and anthrax. Thirty-six cows with various inflammatory diseases were referred to the Veterinary Clinic of Shiraz University, Iran with common infectious diseases such as acute diffuse traumatic reticuloperitonitis (TRP) (8 cases), digestive tract infections and abomasal displacement (6 cases), anaplasmosis due to *Anaplasma marginale* (6 cases), theileriosis due to *Theileria annulata* (6 cases), acute respiratory infections (5 cases) and clinical mastitis (5 cases). Diseased cows were thoroughly examined and samples were collected for haematology, clinical biochemistry and other relevant analysis. The presense of inflammation and disease was assessed on the basis of clinical examination and laboratory findings. Blood samples for determination of serum Hp were collected from the coccygeal vein of both clinically healthy and diseased groups in plain vacutainers and the serum was separated following centrifugation for 15 min at 750 *g*. Serum samples were stored at –20 ^{o}C until analyzed. Hp was measured on the basis of the preservation of the peroxidase activity of haemoglobin which is directly proportional to the amount of haptoglobin using a colorimetric commercial kit (Tridelta Development Plc, Co., Wicklow, Ireland).

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

Serum haptoglobin concentrations (mg/mL) of clinically healthy cattle are presented in Table 1, and those of cows with common infectious diseases – in Table 2. There was no significant difference in Hp concentrations between healthy Holstein cattle of the different age groups as well as between both sexes. Pregnancy and lactation had no significant effects on Hp concentrations. However, significant difference in Hp was observed between clinically healthy and diseased cows (0.20±0.03 mg/mL vs 0.94±0.10 mg/mL, respectively; $P<0.05$). There were also

Table 1. Serum concentrations of haptoglobin (mg/mL) in clinically healthy Holstein cattle

Healthy cows	Number	Mean±SEM
Age, years		
<2	19	0.31 ± 0.04
2-4	19	0.17 ± 0.06
>4	12	0.16 ± 0.06
Sex		
Male	7	0.24 ± 0.07
Female	43	0.22 ± 0.03
Pregnancy		
Pregnant	13	0.08 ± 0.06
Non-pregnant	17	0.22 ± 0.05
Lactation		
Dry	7	0.30 ± 0.09
Dairy	23	0.12 ± 0.05
Total	50	0.20 ± 0.03

significant differences in Hp concentrations between cattle suffering from acute diffuse traumatic reticuloperitonitis, anaplasmosis, theileriosis and acute respiratory infections compared to both those with digestive tract infection and abomasal displacement and clinical mastitis (P<0.05). In diseased cattle, the lowest concentrations of Hp (0.60±0.16 mg/mL) were observed in those with digestive tract infection and abomasal displacement (Table 2).

DISCUSSION

The concentration of Hp in clinically healthy cattle was 0.20 ± 0.03 mg/mL which is in agreement with the findings of others (Salonen *et al*., 1996; Nakagawa *et al*., 1997; Lipperheide *et al*., 1997). Sex, age, lactation and pregnancy had no significant effect on serum Hp of clinically healthy cattle. Bertoni *et al*. (1997) stated that if serum Hp on the 15th day of lactation was more than 1 g/L, early culling of cows should be considered. Taira *et al*. (1992) evaluated the effects of age, parturition and inflammation in horses. The highest concentration of serum Hp was observed in horses under the age of 12 months and it decreased with aging. In pregnant mares, serum Hp was higher 4 months prior to and during parturition and

Table 2. Serum concentrations of haptoglobin (mg/mL) in cows with some common infectious diseases

Diseased cows	Number	Mean ± SEM
Age, years		
<2	7	0.90 ± 0.11
2-4	11	0.88 ± 0.09
>4	18	1.00 ± 0.10
Sex		
Male	–	–
Female	36	0.94 ± 0.10
Diseases		
Acute diffuse traumatic reticuloperitonitis	8	1.08 ± 0.09 [a]
Digestive tract infection and abomasal displacement	6	0.60 ± 0.16 [b]
Anaplasmosis	6	1.02 ± 0.07 [a]
Theileriosis	6	1.11 ± 0.08 [a]
Acute respiratory infections	5	1.09 ± 0.14 [a]
Clinical mastitis	5	0.80 ± 0.12 [c]
Total	36	0.94 ± 0.10

Means within a column with different superscripts (a,b,c) denote significant differences (P<0.05).

decreased 12 weeks postpartum (Taira *et al.*, 1992). In our study, blood Hp in calves was slightly higher in comparison with heifers and adult cows. In cows, Uchida *et al*. (1993) reported increased serum Hp during pregnancy and especially near parturition due to cortisol and non-steroid fatty acids. In the present study, Hp in dry cows near parturition was slightly higher than Hp in dairy cows. Serum Hp in pregnant cows was higher in comparison with non-pregnant ones.

In acute, subacute and chronic inflammations such as pneumonia, peritonitis and abscess, Alsemgeest *et al*. (1994) observed increased Hp and introduced Hp as a valuable marker for differentiating healthy cows from diseased ones. Lipperheide *et al*. (1997) found that serum Hp increased during inflammatory diseases using nephelometry. Gadson *et al*. (1996) evaluated acute phase proteins such as Hp in cows with respiratory infections and introduced Hp as diagnostic factor. Increased serum or plasma Hp concentration in cattle were also reported after trauma (Fisher *et al*., 2001; Earley & Crowe, 2002), experimental local aseptic inflammation (Bremner, 1964; Conner & Eckersall, 1988) and acute infections under field conditions (Skinner *et al*., 1991).

In the 36 diseased cows subject to our study, Hp levels were significantly higher (0.94±0.10 mg/mL) than those in healthy ones (0.20±0.03 mg/mL; $P<0.05$).

Hirvonen *et al*. (1996) found Hp to be the most useful factor in diagnosis of severity and prognosis of mastitis. Salonen *et al*. (1996) and Ohtsuka *et al.* (2001) observed higher serum Hp in cows suffering from *E. coli* mastitis, whereas Eckersall *et al*. (2001) reported elevation of Hp in serum and milk in mild mastitis. Milk and serum Hp increased rapidly in the acute phase of staphylococcal mastitis (Gronlund *et al.*, 2003). Nielsen *et al*. (2004) reported that Hp in cows with clinical mastitis was higher than in those with extramammary infection. Gronlund *et al*. (2005) reported substantial variation in Hp and serum amyloid A concentrations in milk from udder quarters with chronic subclinical mastitis. Our data about cows with clinical mastitis are in accordance with the cited investigations.

Concentration of serum Hp is reduced in haemolytic anemias, but increases following inflammatory conditions (Alsemgeest *et al.*, 1994). Hp is a prominent acute phase protein in most species studied, but its serum concentration can be influenced by other factors besides the acute phase response. Increased levels of free haemoglobin are followed by decreased serum Hp concentrations. In cattle, during an acute hemolytic crisis due to babesiosis (Bremner, 1964), Hp was not detected in the circulation. An effect of free haemoglobin in serum samples towards reduction of measured concentration of Hp has also been found (Eckersall *et al*.,1999; Petersen *et al*., 2001). These data are different from our results in cattle with anaplasmosis and theileriosis, in which we observed increased serum Hp concentrations.

Our study showed that acute respiratory infections resulted in significant increase of serum Hp levels. Similar findings were demonstrated in cattle with experimental infection with *Pasteurella haemolytica* (Katoh & Nakagawa, 1999; Ganheim *et al*., 2003). In calves infected only with *P. haemolytica* serotype Al, no increase in serum Hp concentration was observed within the first 10 hours after inoculation (Horadagoda *et al*., 1994). Experimental induction of pneumonia by inoculation of *P. multocida* has shown that a high challenge volume was associ-

ated with increased plasma Hp concentrations irrespective of the number of bacteria (Dowling *et al*., 2002). In feedlot cattle with clinical respiratory tract disease, a high but variable Hp response was observed (Wittum *et al*.,1996).

Hirvonen & Pyorala (1998) reported increased serum Hp in 55% of cows prior to and 2–3 days after operation of TRP or abomasal displacement. In our study, gastrointestinal tract disorders as acute diffuse TRP, digestive tract infections and abomasal displacement were also found to provoke significantly elevated Hp values. Similar results were communicated in acute inflammation, rumenotomy (Morimatsu *et al*., 1992) and off feeding for 3 days (Katoh *et al.*, 2002). This acute-phase protein was also used to differentiate between healthy and diseased cattle with regard to food safety improvement in farms and slaughterhouses (Saini *et al*., 1998).

The results of the present study revealed that Hp was a sensitive marker in various inflammatory conditions in cattle. The studied parameter could be influenced by other factors such as renal and hepatic disorders, significant changes in haemoglobin levels, so it shoud not be used independently but together with other blood laboratory indices with regard to better elucidation of the systemic status.

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