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Serum Haptoglobin Concentration in Cattle

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ABSTRACT. To obtain a basal concentration of serum Haptoglobin (Hp) in cattle in Taiwan, Hp concentrations were measured from serum samples collected from 10 healthy heifers, every week for one year. The values were also compared with those collected from 15 cows diagnosed with postpartum metritis. The heifers were successfully impregnated by artificial insemination six months after the tests. Hp concentrations were also measured in the serum collected from 11 other cows within 3 weeks after parturition. The Hp assay developed in this study gave a good correlation (r=0.893) with Western blotting. The Hp concentration of 454 serum samples from the 10 heifers had a mean value of 83.6 ± 34.1 mg/l, and there was no significant difference among individual heifers. The basal value of Hp in heifers was calculated as less than 73.6 mg/l. No significant difference in Hp concentration was observed among the 10 heifers during cold and warm seasons (19.8 ± 2.2°C vs 27.3 ± 1.4°C), or before and after pregnancy. The mean serum Hp concentration from cows suffering from postpartum reproductive disorders was 1133.5 ± 627.1 mg/l, which was significantly greater than the serum of healthy heifers and postpartum cows (104.6 ± 61.0 mg/l) (P<0.05). These results demonstrate that Hp concentration may be a useful indicator for cows with postpartum reproductive disorders.

KEY WORDS: basal value, cattle, Haptoglobin.

Acute phase proteins (APP) refer to a group of hepatic glycoproteins which are stimulated by inflammatory mediators and respond to an initial reaction to infection, inflammation or trauma in animals [10]. The function of APP is to promote immunoglobulin production and tissue repair, preventing further injury and recycling useful molecules and debris [7]. C-reactive protein (CRP) is generally not regarded as an acute phase protein in cattle [5]. Haptoglobin (Hp) is one of the most specific APP in cattle [2, 12]. Connor et al. [4] reported that Hp concentration may increase up to 100 fold within 24 hr of induced localized inflammation in cattle. Hp exhibits a significant increase during an acute phase response in both experimental and naturally occurring infections and inflammatory conditions [7, 12, 14]. Hp can be helpful in distinguishing acute from chronic inflammatory diseases [1], although the non-infective conditions of milk fever and ketosis do not produce increased plasma Hp values [13]. In addition, measurement of APP concentration in veterinary diagnostics and treatment, especially as an inspection tool in slaughterhouses for improving food safety, has recently become fully appreciated [11, 16]. All of these findings indicate the usefulness of Hp in the early detection of an acute phase response. To date, no studies have been performed to study the basal value of Hp in heifers, or whether Hp level is influenced by the changing seasons. As such, the aim of this study was to measure serum Hp concentration in healthy cattle and those suffering from postpartum metritis, using a Hp assay with a reagent developed by this laboratory. The seasonal variation of Hp in heifers was also analysed.

MATERIALS AND METHODS

Animals: Between November 2000 and October 2001, thirty-six Holstein cattle from the National Chung Hsing University dairy farm were used for this study. Of these, 10 heifers approximately six months of age and 11 healthy postpartum cows were grouped as clinically healthy animals, while the remaining 15 cows having either retained placenta or postpartum metritis within 3 weeks after parturition were grouped as sick animals. The heifers were in good health and without any underlying pathologic condition. Beginning at one year of age, all 10 heifers were artificially inseminated, followed by successful pregnancies. There was no clinical illness during pregnancy. Clinical examination and blood sampling were performed on the healthy heifers at 1-week intervals for up to one year, on the 11 healthy cows 3 weeks after parturition on the day of diagnosis, and once for the 15 cows during illness. Clinical signs recorded included appetite and rectal temperature.

Western blotting: The concentration of Hp in 20 serum samples was determined by the method of Western blotting in order to estimate the feasibility of the Hp assay developed by our laboratory. Following the separation of Hp by electrophoresis, the gel was blotted with rat antiserum against pig Hp β-chain (donated from the Animal Technology Institute Taiwan; diluted 1:1,000 with TBS containing 3% skim milk) followed by goat anti-rat IgG conjugated with alkaline...
phosphatase (Sigma, A 8438, U.S.A.; diluted 1:4,000). The membrane was then developed with a buffer containing nitro blue tetrazolium and 5-bromo-4-chloro-3-indoly phosphate (Bio-Rad). For quantitative analysis of the constitutive Hp, a total of 20 serum samples and 2 standards were Western blotted and the optical densities of Hp on nitrocellulose membranes were determined using a densitometer (Media Cybernetics, U.S.A.; software was Gel-Pro® Analyzer 4.0).

**Hp analysis:** Blood samples were collected by coccygeal venepuncture and collection in tubes for analysis of serum Hp concentration. In all, 487 serum samples from the heifers, 11 samples from the healthy cows and 15 samples from the cows with illness were centrifuged at 1,000 g for 10 min. The sera were as free as possible of hemolysis and kept frozen (– 20°C) until analysis. Hp was measured by determining the hemoglobin binding capacity (HbBC) of serum according to the method of Chu et al. [3]. Hb was prepared from fresh bovine erythrocytes and diluted with double distilled water to give a working concentration of 0.34 mg/ml. Each analysis used 20 µl of test serum and standards incubated at room temperature with 10 µl of Hb for 10 min, followed by 100 µl of sodium acetate buffer (0.1 M, pH 3.6) to all the wells. The microwells were kept at 37°C for 1 hr, then 140 µl of tetramethylbenzidine (TMB) was added before reading the absorbance at 650 nm. Individual samples were quantified against the standard sample. If the concentration was greater than the range of the standard curve, they were diluted as necessary.

**Statistical analysis:** Interassay comparisons were determined, using analysis of variance and unpaired t-test. Significant difference was not seen among the heifers, therefore, all the sera from the 10 heifers were identified as being a group. Because the Hp values showed an abnormal distribution, all the Hp values were divided into 2 populations after logarithmic transformation. Population A constituted 93.8% (426/454) of all the values and showed a normal distribution. The mean value plus 2 standard deviation (mean + 2 SD) was concluded as being the basal value of Hp in heifers. The statistical difference between cold (Nov. - Apr.) and warm (May - Oct.) seasons was evaluated by chi square test of homogeneity. Significant differences in Hp values between the normal healthy heifers, the normal postpartum cows and cows with postpartum metritis were conducted by Dunnett’s test. The differences were considered significant at P<0.05.

**RESULTS**

The results indicate that there was generally a good correlation (r=0.893) between the Hp values of the 20 serum samples determined by Western blot and the Hp assay in this study (Fig. 1).

Because hemolysis significantly influences the HbBC [3, 11], 33 hemolytic serum samples from the 10 heifers were excluded from the analysis. The average Hp concentration of healthy heifers from a total of 454 serum samples was 83.6 mg/l ± 34.1 mg/l (Fig. 2). The maximum limit of Hp concentration was less than 300 mg/l, which constituted 94.9 % (431/454) of all the values (Table 1). The basal value in heifers was calculated as 73.6 mg/l. Serum Hp concentration did not differ among individual heifers, as well as before and after pregnancy status. In comparing the Hp concentration over the course of the year, there was no significant difference between cold (19.8 ± 2.2°C) and warm (27.3 ± 1.4°C) seasons (P=0.387).

Of the 15 cows with postpartum reproductive disorders, 11 developed fever (> 39.5°C) and poor appetite. The
**HAPTOGLOBIN IN CATTLE**

Table 1. The distribution of detected Hp concentration from the 10 healthy heifers

<table>
<thead>
<tr>
<th>Haptoglobin (mg/l)</th>
<th>Samples</th>
<th>Relative frequency (%)</th>
<th>Cumulative relative frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.0 – 49.9</td>
<td>349</td>
<td>76.9</td>
<td>76.9</td>
</tr>
<tr>
<td>50.0 – 99.9</td>
<td>73</td>
<td>16.1</td>
<td>93.0</td>
</tr>
<tr>
<td>100.0 – 199.9</td>
<td>4</td>
<td>0.8</td>
<td>93.8</td>
</tr>
<tr>
<td>200.0 – 299.9</td>
<td>5</td>
<td>1.1</td>
<td>94.9</td>
</tr>
<tr>
<td>300.0 – 399.9</td>
<td>3</td>
<td>0.7</td>
<td>95.6</td>
</tr>
<tr>
<td>400.0 – 499.9</td>
<td>5</td>
<td>1.1</td>
<td>96.7</td>
</tr>
<tr>
<td>500.0 – 999.9</td>
<td>7</td>
<td>1.5</td>
<td>98.2</td>
</tr>
<tr>
<td>&gt; 1000.0</td>
<td>8</td>
<td>1.8</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Total 454 100.0 100.0

Fig. 3. Comparison of common logarithms of serum Hp concentration between healthy heifers (NH, n=10), cows with normal postpartum (NP, n=11), and postpartum metritis (PM, n=15). The mean Hp concentration of PM (★) was significantly higher than those of NH and NP (P<0.05, Dunnett’s test).

serum samples from these cows had Hp concentrations ranging from 435.3 mg/l to 2880.6 mg/l. The mean serum Hp concentration was 1133.5 mg/l ± 627.1 mg/l, which was significantly higher than those from the 11 healthy postpartum cows (104.6 mg/l ± 61.0 mg/l, ranging from 22.8 mg/l to 194.2 mg/l) and the 10 heifers (83.6 mg/l ± 34.1 mg/l) (P<0.05). The lowest 10% of the Hp concentration from the 15 cows with retained placenta or postpartum metritis was still higher than 95% of those from the normal 454 serum samples (Fig. 3).

**DISCUSSION**

Recently, the monoclonal antibody to bovine Hp has been used to measure Hp values in bovine sera using an enzyme-linked immunosorbent assay. However, such a rapid ELISA procedure is too expensive to perform as a frequent routine bovine Hp screening test which could provide early warning of infection [16]. Therefore, Young et al. [16] further developed three different immunoassay formats for bovine Hp. Incorporating Hb binding was considered to be a precise and reproducible method for bovine Hp detection. Hp levels determined by the Hp assay in the present study correlated with those by Western blot (r=0.893). Therefore, the rapid Hp assay described herein was shown to be simple and inexpensive for field use.

There was no significant difference between Hp values in cold and warm seasons (19.8 ± 2.2°C vs 27.3 ± 1.4°C) in our data. This result indicates that sampling for Hp measurement can be performed at any time during the year. Because sex and age of cattle have no discernible effect on Hp concentration [7, 11], healthy heifers, which were regarded free from physical stress such as lactation and pregnancy, were used in this study to obtain normal Hp values in cattle. Saini et al. [11] reported that lactation or pregnancy in cattle appeared to have no effect on Hp concentration. In the present study, the results, which were consistent with the results reported previously, showed serum Hp concentration did not differ among individual heifers, and did not differ between before and after pregnancy. The CRP level is significantly elevated during pregnancy [8], therefore, Hp is considered to be suitable as a major acute phase protein in cattle.

The heifers were in a generally healthy condition, and had mean and median Hp concentrations of 83.6 mg/l and 35.3 mg/l, respectively. These values, however, were disparate and were not located in the majority of the population. Therefore, we analyzed all the Hp values with logarithmic transformation, and the basal value was finally determined to be 73.6 mg/l, while values higher than 73.6 mg/l were considered representative of a population which exhibited clinically normal conditions but under potential stress. However, Salonen et al. [12] reported previously that a mean Hp concentration of 35 mg/l was detected from only 8 serum samples of healthy cows by high performance liquid chromatography, and 12 ± 5 mg/l has also been reported as a normal value of Hp from 20 serum samples of cattle by using commercial kits (Technicon Ltd) for Hp assay [13]. Both values are much lower than our normal value. The difference may be attributable to the fact that the experiments were performed with different laboratory assay methods and sample sizes.

Skinner et al. and Kent have reported that non-inflammatory diseases such as milk fever and ketosis, nutritional status, exercise, and minor stress should not affect the values of acute phase proteins [7, 13]. Even though stress may not stimulate an acute phase response, there was evidence that it had a role in activating pre-existing latent infection, and resulting in an acute phase response [11]. Uchida et al. suggested that only a negligible or low basal value of Hp was detectable in normal bovine serum, but Hp could be induced by an acute phase response such as inflammation, transportation, exhaustion, stress or starvation [15]. Additionally, Hp was detected in 31 of 42 cows (73.8%) within one day after parturition, and it was also suggested that Hp was associated with fatty liver development in cows during the peripartum period [15]. Skinner et al. concluded that a Hp concentration of > 0.4 g/l may indicate acute bacterial infec-
tion in cattle, and that > 0.2 g/l may indicate early or mild infection [13]. In this study, only a few samples from apparently normal heifers showed a high Hp concentration and they may represent cases with previous or mild inflammatory conditions, or severe stress despite the absence of clinical signs. It should be noted that week, sampling was performed 3 days after herd vaccination. The Hp value was remarkably increased in 7 of the 10 heifers, and then reverted to a normal range again the following week. This result supports the theory that high Hp concentration beyond the threshold value may be a particularly useful early surveillance method for cattle diseases.

It was reported that the serum Hp concentration level was remarkably high in cattle suffering from severe mastitis or postpartum metritis [9, 13]. Of the 15 cows in our study with retained placenta or postpartum metritis, the mean serum Hp concentration was 1133.5 mg/l ± 627.1 mg/l, which was exceedingly higher than those from healthy heifers and postpartum cows (P<0.05). In addition, 10% of the lowest Hp concentration from the 15 cows with postpartum reproductive disorders was higher than 95% of all the serum samples from healthy heifers and postpartum cows. It is concluded that Hp concentration is a useful indicator for surveying some reproduction problems in cattle after parturition. However, further study with larger numbers of samples and more confirmed diseased conditions will be necessary to establish the relationship between bovine postpartum reproductive disorders and changes in serum Hp concentration. The usefulness of serum Hp concentration can then be evaluated for the early detection of certain bovine postpartum reproductive disorders.

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REFERENCES