Acute-phase Proteins and Interleukin-6 (IL-6) in Cattle Infected with Subclinical Parasitic Infections

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ABSTRACT

Background: The acute phase response (APR) has been well documented for clinical infections. There are relatively few reports regarding the potential uses of acute phase proteins (APP) for the recognition of subclinical infection. Until now, there have been no reports published on the development of the APR in subclinical infection with parasites. Haptoglobin (Hp) and serum amyloid A (SAA) are important APPs for cattle. Interleukin-6 (IL-6) is considered a major immune and inflammatory mediator and the principal regulator of most APPs. This study was carried out to ascertain the levels of SAA, Hp and IL-6 in cattle infected with low levels of various parasites.

Materials, Methods & Results: Thirty six slaughtered cattle (20 male, 16 female) of varying ages were used. Clinical findings such as pain, redness, slackness, diarrhoea or fever were not seen in these cattle. Blood samples were taken into vacutainers without heparin during slaughter, the internal organs of the cattle were examined postmortem for possible infections and faecal samples were collected. For detection, Benedict’s sedimentation was used for trematode infections, saturated water flotation was used for cestode and nematode infections, and the Baermann - Wetzel method was used for lungworm infections. A total of 28 cattle with parasitic infection were evaluated as the study group and eight parasitologically free cattle were used as the control group. In the study group, 12 of the 28 cattle were infected with Eimeria sp. only, 10 with Trichostrongylidae only, one with hydatid cysts only and five with mixed parasites (Trichostrongylidae + Eimeria sp. in one animal, Trichostrongylidae + hydatid cyst in two animals, Trichostrongylidae + Moniezia sp. in two animals). Trichostrongylidae and Eimeria sp. infections were found at low levels. Serum amyloid A, Hp and IL-6 levels in the serum samples were analysed using the enzyme - linked immunosorbent assay method. In the present study, levels (mean ± standard error) of SAA and Hp were significantly ($P < 0.01$) lower (1.83 ± 0.52 µg / mL and 0.77 ± 0.15 ng / mL, respectively) in the study group compared with those of the control group (4.84 ± 0.51 µg / mL and 3.87 ± 0.91 ng / mL, respectively). The serum concentration of IL-6 in the study group was significantly ($P < 0.01$) higher than that in the control group. This elevated level was higher in cattle infected with Eimeria sp. and mixed parasites ($P < 0.01$). Serum SAA levels of cattle infected with Eimeria sp. and Trichostrongylidae were statistically lower compared with those of the control group ($P < 0.05$).

Discussion: Acute phase proteins are specific indicators of tissue damage. Therefore, these proteins are an important parameter in terms of herd health and the monitoring of treatment during infections in veterinary medicine. Subclinical parasitic diseases are an important cause of production losses in cattle, even though this condition is very often found in field conditions. In the present study, Hp and SAA levels were significantly lower and the level of IL-6 significantly higher in cattle infected with a low level of parasites when compared with healthy animals. The determination of serum Hp and SAA concentrations may be helpful clinically to monitor concomitant immunity and to evaluate animal health. The results indicate that measurement of SAA, Hp and IL-6 concentrations may be a valuable in combination for monitoring cattle infected with subclinical parasitic infections.

Keywords: serum amyloid A, haptoglobin, interleukin-6, parasitic infections, cattle.
INTRODUCTION

Following non-specific inflammatory reactions, the acute-phase response (APR) occurs in the body and this response includes changes in the concentrations of plasma proteins known as acute-phase proteins (APPs) [10].

APP synthesis is induced by the release of pro-inflammatory cytokines such as interleukin (IL)-1, IL-6 and tumour necrosis factor– from macrophages and monocytes at the inflammation site [22,34]. IL-6 is considered a major immune and inflammatory mediator [9] and the principal regulator of most APPs [9,40].

APPs are an important parameter in terms of herd health, the diagnosis and prognosis of various diseases, and the monitoring of treatment during infections in veterinary medicine [4,11,16,31].

The APR has been well documented for clinical infections. Hp and SAA are important APPs for cattle, and their serum concentrations increase in hosts with toxic [10,17], parasitic [6,10,17], bacterial [16,29,31] and viral [10,21,31] diseases. There are relatively few reports regarding the potential uses of APPs for the recognition of subclinical infection [26,32,38].

Parasitic diseases are an important cause of production losses in cattle [30] and the pathogenetic effect of these infections may be subclinical or clinical. Until now, there have been no reports published on the development of the APR in subclinical infection with mixed parasites, even though this condition is very often found in field conditions. Therefore, the aim of this study, serum concentrations of SAA, Hp and IL-6 in apparently healthy cattle, but with parasitic infections, were determined.

MATERIALS AND METHODS

Selection of Animals and Collection of Samples

In the study, 36 slaughtered cattle (20 male, 16 female) of varying ages were used. Clinical findings such as pain, redness, slackness, diarrhoea or fever were not seen in these cattle.

Blood samples were taken into vacutainers without heparin during slaughter, the internal organs of the cattle were examined postmortem for possible infections and faecal samples were collected.

Following the postmortem and faecal examinations, 28 cattle were diagnosed with parasitic infection and were evaluated as the study group; eight cattle that were not diagnosed with any parasitic infections were evaluated as the control group. Before the start of the study the cattle were allocated to subgroups according to various parasitic infections.

Parasitological Procedure

All faecal samples were first examined macroscopically, and then they were examined microscopically. For detection, Benedict’s sedimentation was used for trematode infections, saturated water flotation was used for cestode and nematode infections, and the Baermann–Wetzel method was used for lungworm infections. In faeces found positive for gastrointestinal nematodes and Eimeria sp., egg number (eggs per gram; EPG) and oocyst number (oocysts per gram; OPG) were determined per gram faeces using the McMaster method to determine the severity of infection [20,39].

Serological Tests

Serum amyloid A (SAA) [CSB-E08592b]1, Haptoglobin (Hp) [CSB-E08585b]1 and interleukin-6 (IL-6) [CSB-E12899b]1 levels in the serum samples were analysed using the enzyme-linked immunosorbent assay (ELISA) method.

Statistical Analysis

The t test was used to analyse the significance of the difference between the study and control groups. One-way analysis of variance and Duncan’s test were used to evaluate the differences between the infected subgroups in the study group (SPSS 13.0). Results are given as mean ± standard error. In all cases, a probability of error less than 0.05 was selected as the criterion for statistical significance.

RESULTS

In the study group, 12 of the 28 cattle were infected with Eimeria sp. only, 10 with Trichostrongylidae only, one with hydatid cysts only and five with mixed parasites (Trichostrongylidae + Eimeria sp. in one animal, Trichostrongylidae + hydatid cysts in two animals, Trichostrongylidae + Moniezia sp. in two animals).

The number of eggs (EPG) and OPG in the faecal samples determined to contain Trichostrongylidae eggs and Eimeria sp. oocysts were 25-650 and 50-300, respectively.
SAA concentrations in serum were 1.83 ± 0.52 and 4.84 ± 0.51 µg/mL in the study and control groups, respectively (P < 0.01). Hp concentrations in serum were 0.77 ± 0.15 and 3.87 ± 0.91 ng/mL in the study and control groups, respectively (P < 0.01). However, the serum IL-6 level in the study group was 311.52 ± 41.32 pg/mL, and 83.02 ± 17.87 pg/mL in the control group (P < 0.01) [Table 1].

For the study group, in cattle infected with Trichostrongylidae, *Eimeria* sp. and mixed parasites, serum SAA, Hp and IL-6 levels are shown in Table 2. In the subgroups of cattle infected with these parasites, serum IL-6 level was higher than that of the control group (P < 0.01). This elevated level was higher in cattle infected with *Eimeria* sp. and mixed parasites (P < 0.01).

The serum SAA level of cattle infected with *Eimeria* sp. and Trichostrongylidae was significantly lower than that of the control group (P < 0.05). However, the lower SAA concentration in cattle infected with mixed parasites was not significantly lower than that of the controls. Serum Hp levels were lower in cattle infected with Trichostrongylidae, *Eimeria* sp. and mixed parasites compared with those of the control group (P < 0.001).

### Table 1. Concentrations of interleukin-6 (IL-6), serum amyloid A (SAA) and haptoglobin (Hp) in cattle infected with parasitic infections compared with the control group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Study group</th>
<th>Control group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/mL)</td>
<td>311.52 ± 41.32</td>
<td>83.02 ± 17.87</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>SAA (µg/mL)</td>
<td>1.83 ± 0.52</td>
<td>4.84 ± 0.51</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Hp (ng/mL)</td>
<td>0.77 ± 0.15</td>
<td>3.87 ± 0.91</td>
<td>&lt;0.01*</td>
</tr>
</tbody>
</table>

Values are means ± standard errors; *Concentrations of IL-6, SAA and Hp differ significantly (P<0.05).

### Table 2. Concentrations of interleukin-6 (IL-6), serum amyloid A (SAA) and haptoglobin (Hp) in infected cattle according to parasite species compared with the control group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group</th>
<th>Study group</th>
<th>Eimeria sp.</th>
<th>Trichostrongylidae</th>
<th>Mixed infections</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/mL)</td>
<td>83.02 ± 17.87</td>
<td>303.47 ± 60.45</td>
<td>179.82 ± 55.58</td>
<td>472.80 ± 82.28</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>SAA (µg/mL)</td>
<td>4.84 ± 0.51a</td>
<td>1.45 ± 0.40b</td>
<td>2.32 ± 1.06b</td>
<td>3.11 ± 1.07ab</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>Hp (ng/mL)</td>
<td>3.87 ± 0.91a</td>
<td>0.65 ± 0.18b</td>
<td>1.03 ± 0.30b</td>
<td>0.56 ± 0.08b</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± standard errors. Means with different letters in a row differ significantly (P<0.05). *Mixed infections Trichostrongylidae + Eimeria sp.; Trichostrongylidae + hydatid cyst; Trichostrongylidae + Moniezia sp.*

### DISCUSSION

APPs are specific indicators of tissue damage [19]. Hp and SAA are higher in acute infections compared with chronic ones [1,24,29] and reach peak levels in the first week after infection and decline to normal levels in the second week [12]. In the present study, the blood levels of the cattle were not evaluated in terms of bacterial or viral infections, as our facilities were insufficient for these examinations. However, it has been suggested that SAA and Hp are more useful in distinguishing acute and chronic inflammation than haematological tests (neutrophil counts and white blood cell counts) [24,35,36]. Therefore, our study cattle were chosen according to anamnestic, clinical findings and postmortem examination. However, future studies should assess bacterial and viral infections in the blood in order to eliminate systemic infection. Clinical symptoms have been associated with higher levels of APPs [6,17]. In our study, no clinical findings were observed in any of the infected cattle. Such animals cannot be easily discerned in a farm population.

The immune responses of hosts to helminth infection are generally characterized by a skewed Th2-like response [27]. IL-6 is discharged from Th2 cells [15,28] and is the principal regulator of most APPs [9,40]. If the cattle in the present study had been suffering from...
bacterial or viral infections, Hp or SAA concentrations could have been higher. The APR is strong in bacterial infections [1], but is milder in viral infections [1,23].

There are relatively few studies of APP induction by parasitic infections in cattle, although infections with Rhipicephalus microplus [3], Anaplasma marginale [6], Theileria annulata [13,18], Cryptosporidium parvum [14], Dictyocaulus viviparus [17] have been reported. In previous studies [1,16,26,33,38], higher APP concentrations were observed in animals with subclinical inflammatory disorders and infected with bacteria or viruses. In the present study, Hp and SAA levels were significantly lower and the level of IL-6 significantly higher in cattle infected with a low level of parasites when compared with healthy animals. According to the present results, there is chronic infection in affected cattle. The result is in agreement with the OPG and EPG findings in faeces. Most significantly, the pro-inflammatory cytokine IL-6 was still activated in these cattle. Inflammation may also develop due to non-infectious causes. Any tissue damage during these processes leads to the release of pro-inflammatory cytokines [40]. The present study demonstrated that the determination of Hp and SAA serum concentrations may be helpful clinically in the monitoring of concomitant immunity. It is known that concomitant immunity can offer an important potential benefit to established worms by preventing overcrowding within the host [2]. In the present study, it was also shown that concentrations of IL-6 were markedly higher at low levels of parasitic infection. However, concentrations of SAA and Hp were lower at low levels of parasitic infection. Cytokines play a major role in the controlling of parasitic infection and can help in the expulsion of adult worms [5]. Additionally, subclinical infections increase the levels of the pro-inflammatory cytokines [25]. Moreover, several experimental models suggest a protective role for IL-6 and specific APPs against inflammation [9]. Therefore, the use of SAA, Hp and IL-6 may be valuable in combination for monitoring cattle infected with parasites.

The level of IL-6 in the present study was higher in the cattle infected with Eimeria sp. only and mixed parasites. The development of Eimeria infection in the host is intracellular [7]. The higher IL-6 concentration in the present study may be due to the life cycle of Eimeria. The IL-6 levels of the mixed infection group were higher than those of the group infected with Trichostrongylidae only. This condition may be connected with the life cycle of Eimeria sp. and hydatid cyst infection. In hydatid cyst infection, the severity of the reaction has been found to be directly related to the infected organ, and to the biological mass size of the larval form. Survival of the parasite causes tissue destruction in infected organs [37].

Horadagoda et al. [24] found that SAA showed the highest sensitivity, while Hp had the highest specificity. In the present study, the SAA levels of cattle infected with Eimeria sp. and Trichostrongylidae were statistically lower than those of the controls. This lower concentration in mixed parasitic infections was not significantly different from that in control cattle. However, Hp levels were lower in cattle infected with Trichostrongylidae, Eimeria sp. and mixed parasites. It is known that most Trichostrongylidae species are localized in the abomasum. In a study conducted by Dilda et al. [8], SAA and Hp are discharged from the abomasum. According to the findings in the present study, Trichostrongylidae infection is at a chronic stage in cattle. Although young cattle are the most susceptible age group for eimeriosis, older animals are generally protected from disease by immunity [7]. In the present study, the cattle infected with Eimeria sp. and Trichostrongylidae were aged more than 1 year old. Thus, the lower levels of SAA and Hp may be related to immunity.

CONCLUSION

The changes in APP values in animals infected with various diseases may be useful in attempting to identify animals that are clinically acutely or chronically infected. In the present study, there were significantly lower concentrations of Hp and SAA together with a significantly higher concentration of IL-6 in cattle infected with low levels of parasites. The determination of serum Hp and SAA concentrations may be helpful clinically to monitor concomitant immunity and to evaluate animal health. The use of SAA, Hp and IL-6 may be valuable in combination to monitor cattle infected with subclinical parasitic infections.

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Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.
REFERENCES


