Interleukins and Acute Phase Proteins of Bovine Sera during Natural Helminth Burden in Ibadan Nigeria

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Abstract

Inflammatory reactions in the gastrointestinal tract play an important role in the pathogenesis of gastroenteritis in bovine helminthosis. This study determined the serum concentrations of cytokines induced acute phase proteins (Serum Amyloid A (SAA), Haptoglobin (Hp) and C-reactive protein (CRP)) and the levels of immunoreactive interleukins (IL-1, IL-2, IL-3, IL-4, IL-5 and IL-6) in cattle naturally infected with helminths. We sampled a total of 480 slaughtered cattle of both sexes in a major abattoir in Ibadan, Nigeria. Sedimentation, floatation and modified McMaster techniques were employed to determine the degrees of helminth infections. Animals with eggs per gram (epg) less than 200 were adjudged to be apparently healthy. The serum concentrations of interleukins and acute phase proteins were determined using the Technicon AutoAnalyzer Model AA II. It was found that mean SAA (µg/l) and CRP (%) levels were significantly (P < 0.05) higher in cattle with helminthosis as compared to values measured in the apparently normal animals. In addition, SAA and Hp levels in the helminth positive cases correlated with the increase in immunoreactive interleukin-6. However, the analyses of the results between the evaluated groups did not show any significant differences in serum concentration of Hp (µg/l) (P > 0.05). Therefore, some acute phase proteins are involved in the pathophysiology of bovine helminthosis and are closely related to the inflammatory activation of the disease. In lieu of these findings, it is suggested that systemic markers of inflammation can identify subjects at high risk of natural bovine helminthosis and that IL-6 and SAA may be used as indicators for bovine helminthosis.

Keywords

C-Reactive Protein (CRP), Interleukins, Bovine Helminthosis, Haptoglobin, Serum Amyloid A (SAA)
1. Introduction

Helminth infections are ubiquitous and remain a constraint to the efficient raising of cattle on pasture. The invasion of a host by gastrointestinal worms is usually accompanied by some mechanical damage to the tissues [1].

Acute phase proteins (APPs) are blood proteins synthesized by hepatocytes as part of the innate immune system’s response to various stimuli such as inflammation, trauma, and infection [2]. The main function of APPs is to assist in defending the host against pathological damage and to restore homeostasis [3]. These APPs are used in the early and accurate detection of infections in ruminants [4]. Applications of acute phase proteins (APPs) in bovine medicine have largely focused on diseases in which the acute phase response would be expected from the known involvement of infection, inflammation and the stimulation of cytokine driven responses. Alteration in APP expression has been associated with a number of food animal diseases such as mastitis, metritis, and amyloidosis in lactating dairy cattle [5] [6]. In the clinical field, investigation of the APP response in natural cases of disease is considered as an indicator of sub-clinical disease, prognosis and effect of treatment in cattle [7] [8]. The acute phase response develops in a wide range of diseases. In cattle, elevated blood serum concentrations of haptoglobin and SAA, as two major acute phase proteins, have been demonstrated in association with several diseases [9]. These conditions cause release of interleukin-6 and other cytokines that trigger the synthesis of acute phase proteins by the liver. The serum levels of acute phase proteins might serve as surrogates for IL-6 activity in vivo [10].

Serum concentrations of positive APPs, such as haptoglobin (Hp), C-reactive protein (CRP) and serum amyloid A (SAA) increase during acute phase response (APR) [11].

In Nigeria, many studies have been conducted on helminth infection in slaughtered and field cattle particularly its effects on health, erythrocyte index, blood glucose and age of susceptibility in affected animals among others [12] [13] [14] [15]. There seems to be a dearth of information on the use of interleukins and acute phase proteins as indicators of bovine helminthosis in Nigeria. This study therefore aims to determine changes in the levels of cytokines induced acute phase proteins [Serum Amyloid A (SAA), Haptoglobin (Hp) and C-reactive protein (CRP)] and the levels of immunoreactive interleukins (IL-1, IL-2, IL-3, IL-4, IL-5 and IL-6) in cattle naturally infected with helminth worms and apparently healthy ones and therefore to provide a baseline data in contribution to metabolomics in large ruminant internal medicine.

2. Materials and Methods

2.1. Animals

The study was performed over a period of six months at the Bodija Central Abattoir, Ibadan Southwestern Nigeria. Most cattle are brought for slaughter in this abattoir from the northern parts of Nigeria. All the animals to be sampled
were clinically examined and found to be free from any obvious clinical signs of inflammation such as vaginitis, metritis, balanitis, arthritis, claw disorder, lameness, orchitis, pneumonia, mastitis and transportation stress. Blood and faecal samples were obtained from 480 cattle of both sexes, and of different ages. Breeds of cattle sampled included 284 White Fulani cattle, 76 Red Bororo cattle, 20 Sokoto Gudali cattle, 8 Kuri cattle, and 92 crosses of some of the breeds.

2.2. Screening of Helminth Eggs in Bovine Faecal Samples

Faecal sample was collected from the rectum of each of 480 cattle into labeled 12.5 × 12.5 cm polythene bags and held at 4˚C in a cool box before analysis in the Parasitology Laboratory of Department of Veterinary Microbiology and Parasitology at the University of Ibadan, Nigeria. Samples were screened for the presence of helminthes by employing sedimentation, floatation and modified McMaster techniques according to the methods described by earlier workers [16] [17]. Helminth eggs were detected quantitatively with a sample dilution of 15 ml salt solution/g faeces and 30 mins flotation time. Using a disposable pipette both chambers of the McMaster slide (Weber scientific using Instruments, Middlesex, England) were filled with the suspension. The slide was allowed to stand on the bench for five minutes and all of the eggs under the grid areas of the two chambers were counted. Examinations were carried out using a stereoscopic microscope at x40 magnification and hand-held tally counter used to record the number of eggs present. Morphological features were used to identify the different helminth eggs according to the methods described by an earlier scientist [18]. The animals were thereafter categorized into two groups according to the presence or absence of helminth eggs in faecal samples as earlier described elsewhere [19]. The degree of helminth infection was determined from the egg per gram count of faeces.

2.3. Serum Sample Analysis

At slaughter, about 5 ml of blood was collected from the jugular vein of each animal into sterile well-labelled vacutainer tube (Beckton Dickson®, France) and transported in ice-boxes to the general laboratory of Department of Veterinary Medicine, University of Ibadan Nigeria. A drop of each blood sample was placed on a glass slide and processed for laboratory examination for the presence of Babesia, Trypanosome organisms and other blood parasites, using standard laboratory techniques. Only blood samples that were negative for the haemoparasites were selected for serum analysis. Each serum was then separated by centrifuging the blood samples at 5000 rpm and kept at −20˚C until analyzed. Serum samples were analyzed for the estimation of acute phase proteins (APPs) and interleukins using Technicon AutoAnalyzer Model AA II (Bran and Leubbe Pty Ltd., Homebush, New South Wales, Australia) according to techniques earlier described by other researchers [20]. Digestion of serum samples was carried out.
using 0.15 ml of sample in a digestion tube with 1 g of Se/Kaydel tablet, 5 mls of sulphuric acid, and 2.5 mls of Na-dichloroisocynurate for 1 hour 30 minutes at 150°C and cooled to room temperature. The contents were transferred to centrifuge tubes, vortexed and centrifuged at 5500 rpm for 15 mins. The digest was then transferred to a set of glass vials instead of plastic vials that could cause contamination. All these steps were taken in accordance with what other workers had done [21].

2.4. Quantification of Acute Phase Proteins (CRP, Hp and SAA) in Serum Samples

The serum levels of C-Reactive Proteins, Haptoglobin and Serum Amyloid A were estimated using the autoanalyzer. There was the development of colour specific for the analyte of interest at a specific wavelength (without any formula for calculations, because it is an automatic machine). C-reactive protein ran at a wavelength of 650 nm with a conversion factor of 6.25 while Haptoglobin ran at a wavelength of 713 nm with a conversion factor of 4.86 and Serum Amyloid A ran at a wavelength of 436 nm using a conversion factor of 5.2.

2.5. Quantification of Some Interleukin Classes in Sera

The serum levels of IL-1, IL-2, IL-3, IL-4, IL-5 and IL-6 were estimated using 2 ml of sodium-dichloroisocyanurate, 1.5 ml of salicylic acid, (but 15 ml of salicylic acid for IL-6) and 10 ml of ultra-pure water. Solution for each interleukin class was shaken for 5 minutes and allowed to run in the autoanalyzer at wavelengths of 238 nm, 358 nm, 189 nm, 157 nm, 480 nm and 644 nm respectively. For calibration, a standard solution of 0 to 8 ppm was used for all the reactions.

2.6. Data Analyses

Statistical analyses were performed to assess the pattern of changes and the relative values of serum parameters and to find a possible relationship between interleukins and acute phase proteins (APPs) and their changes in natural bovine helminthosis.

All statistical analyses of the data were completed using IBM Statistical Package for Social Sciences (SPSS) software package version 21 and GraphPAD PRISM Version 4.00 (San Diego, CA, USA). Data were expressed as mean and standard deviation (SD). Confidence level was held at 95% and values of p < 0.05 were considered statistical significant.

Distribution pattern of data was assessed by Kolmogorov-Smirnov test (p < 0.05). Comparisons between means were made by using the Mann-Whitney U test. Spearman correlation coefficient was calculated to access the association among the variables studied.

Spearman correlation was used to access the association among the variables studied especially the relationship between interleukin-6 and acute phase proteins.
3. Results

3.1 Two Groups of Sampled Cattle

The two categories of sampled cattle based on the animals with zero to 200 eggs per gram faeces (epg) and those with more 200 epg are shown in Table 1. It is observed that 176 cattle were found to have more than 200 epg while 304 of them were found to have between zero and 200 epg. In the order of decreasing occurrence, the bovine nematodes encountered included *Haemanchus placei*, *Oesophagostomum radiatum*, *Ostertagia ostertagi* and *Syngamus laryngeus*.

3.2. The Acute Phase Proteins

Figure 1 shows the mean, standard deviation (SD) and P-values of the selected acute phase proteins in apparently healthy cattle and cattle infected with bovine helminthosis. The serum concentrations of CRP and SAA were significantly higher (P < 0.05) in cattle that were positive for bovine helminthosis (14.14% ± 0.67%, 3.43 ± 1.83 µg/l respectively) as compared with apparently healthy animals (13.45% ± 1.03%, 2.40 ± 0.86 µg/l respectively). There was no difference in mean concentrations of Haptoglobin (Hp) between the two groups of cattle (1.06 ± 0.67 µg/l and 1.06 ± 0.36 µg/l). Among the three acute phase proteins, the concentration of CRP was highest relatively while that of Hp was the lowest in the cattle populations examined.

Table 1. Two groups of sampled cattle according to eggs per gram (epg) range.

<table>
<thead>
<tr>
<th>No. of cattle</th>
<th>Eggs per gram (epg)</th>
<th>Status (Bovine helminthosis)</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>304</td>
<td>0 - 200</td>
<td>Negative</td>
<td>152.2 ± 51.1</td>
</tr>
<tr>
<td>176</td>
<td>&gt; 200</td>
<td>Positive</td>
<td>810.0 ± 476.7</td>
</tr>
</tbody>
</table>

Figure 1. Serum concentrations of C-reactive protein (CRP), serum amyloid A (SAA) and Haptoglobin (Hp) in the two groups of cattle (apparently healthy and helminth-infected cattle). *P < 0.05.
3.3. The Selected Interleukins

**Figure 2** shows the mean and standard deviation (SD) values of some classes of interleukins in the two groups of cattle (cattle with zero to 200 eggs and those with more than 200 epg). Among the interleukins analyzed, IL-6 response was highest in helminth-infected and apparently healthy cattle (5.36 ± 4.19 µg/l and 2.66 ± 1.16 µg/l respectively). However, no significant differences were observed in the serum concentrations of IL-1, IL-2, IL-3, IL-4, and IL-5 in the two groups of cattle sampled (P > 0.05).

In **Figure 3**, it is obvious that there was significant difference (P > 0.05) in the values of serum concentration of IL-6 in helminth-infected and cattle having between zero and 200 epg with 5.36 ± 4.19 µg/l and 2.66 ± 1.16 µg/l respectively.

There was a strong correlation between the total production of IL-6, SAA and Hp as depicted by the value of Spearman’s correlation coefficient, r. As the level of IL-6 increased in sera of helminth-infected cattle, a significant increase was observed in SAA (r = 0.82, P < 0.01) and Hp (r = 0.70, P < 0.01) levels, suggesting a strong relationship among IL-6, SAA and Hp. In contrast, no significant increase in CRP level was observed as IL-6 and SAA level increased, as shown in **Table 2**.

**Figure 2.** Serum concentrations of IL-1, IL-2, IL-3, IL-4, IL-5 and IL-6 in the two groups of cattle (apparently healthy and helminth-infected cattle).

**Figure 3.** Serum concentrations of IL-6 in the two groups of cattle (cattle with zero to 200 eggs and those with more than 200 epg). *P < 0.05.
Table 2. The summary statistics for correlations among CRP, Hp, SAA, and interleukins levels.

<table>
<thead>
<tr>
<th></th>
<th>CRP (%)</th>
<th>SAA (µg/l)</th>
<th>Hp (µg/l)</th>
<th>IL-1 (µg/l)</th>
<th>IL-2 (µg/l)</th>
<th>IL-3 (µg/l)</th>
<th>IL-4 (µg/l)</th>
<th>IL-5 (µg/l)</th>
<th>IL-6 (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.552*</td>
<td>-</td>
<td>-</td>
<td>-0.473*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SAA (µg/l)</td>
<td>-</td>
<td>-</td>
<td>0.808**</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.820**</td>
<td>-</td>
</tr>
<tr>
<td>Hp (µg/l)</td>
<td>-0.808**</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.700**</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

4. Discussion

In this study, different breeds of cattle and some of their crosses were encountered at the study location. Earlier study by other workers [14] lends credence to the fact that different breeds of cattle and their crosses are brought from the northern parts of the country (Nigeria) into the abattoir where the study was undertaken. The implication of this is that frequent occurrence of crosses of different breeds of cattle is widespread in Nigeria. Therefore, breed cannot be taken into consideration since in Nigeria, exists a lot of crossbreeds of cattle. It has been observed that faecal egg output in cattle is generally low and faecal egg counts are considered to be poor indicator of helminth infection in adult cattle [22]. This assertion has also been confirmed by the number of cattle that were found positive for helminthosis in the present study using faecal egg counts. Although the epg is low across all the sampled cattle, some helminths such as Fasciola or Paramphistome can be harmful to their hosts. Therefore, a baseline study will require that all the animals sampled should be negative for helminths. In this study, selection of animals that were free from apparent clinical inflammatory diseases, haemoparasites and transportation stress, is also very important. Of particular interest is the fact that Trypanosomes and Babesia organisms can cause cardiovascular events and can be powerfully predicted by the production of acute phase proteins [23]. Also, it has been stressed by several workers that acute phase proteins are synthesized in response to cytokines released from macrophages due to tissue damage, infection, inflammation and bacterial components and also after stress [24] [25].

In mammals, the magnitude and duration of the acute phase protein response reflect the severity of the infection and underlying tissue damage [26]. Among different APPs in general, only Hp and SAA have been more extensively investigated in various diseases and inflammatory conditions in cattle. The wide nature of acute phase protein response can be seen as a disadvantage in that the APP assay is not specific for one disease, but this may be associated with various infections and inflammatory processes [27] [28]. In this study, there was a significant increase in the concentrations of SAA and CRP in cattle with helminthosis compared to those cattle that were apparently healthy. However, there was no marked difference between the concentrations of Hp in these two groups of cattle.
These findings are in consonance with the observation of some earlier workers who found out that SAA is a more sensitive acute phase protein than Hp in cattle, with rapid increase in serum concentrations after the inflammatory stimulus [29]. The similarity in the mean concentrations of haptoglobin in cattle with helminthosis and healthy animals might be a consequence of starvation and stress associated with road transport. This observation agrees with the work of [30] that haptoglobin is also induced in cows with fatty liver syndrome, starvation, and following stress associated with road transport. Stress has been found to be sufficient to increase IL-6 levels [31]. Stress in instances of negative life events could increase IL-6 levels as stress might cause alterations in synaptic neurotransmission of adrenaline, norepinephrine or glutamate. Subsequently, astroglia or microglia might be stimulated [32] and induce increased IL-6 production. Activation of IL-6 is closely related to activation of hypothalamic pituitary axis (HPA) [33] the latter being known to be involved in depression and in the stress response [31].

Also, there were no significant increases in the concentrations of IL-1, IL-2, IL-3, IL-4 and IL-5 among the two groups of sampled cattle, except the concentration of IL-6. It has been said that IL-6 is a multifunctional, pleotropic factor, ciliary neurotrophic factor and oncostatin-M and it is involved in regulation of immune response, acute-phase responses, haematopoiesis and inflammation [34]. The difference in acute phase response in the two groups of cattle might be as a result of different initiation of the production of various acute phase proteins as their synthesis is triggered by tumor necrosis factor (TNF)-α, interleukin (IL)-1 and IL-6, demonstrating that T CD4⁺ lymphocytes can produce IL-6 and TNF-α [35]. There are also indications that the response to chronic compared to acute inflammation varies from one protein to another [36]. IL-6 also actively regulates acute and chronic inflammatory processes [37].

5. Conclusions

The findings in this study suggest that natural bovine helminthosis is associated with an acute phase protein response, which is possibly caused by changes in cytokines in infected animals. Therefore, the higher circulating levels of interleukin-6 and significant increase in SAA in cattle with helminth infection, is associated with natural bovine helminthosis.

Measuring SAA has a higher sensitivity compared to CRP and Hp (by correlation). This measure may be useful for identification of high-risk subgroups for anti-inflammatory interventions. It therefore serves as a suitable biochemical marker of natural bovine helminthosis. The various values of some classes of interleukins and acute phase proteins obtained for the apparently healthy and helminth-infected cattle cannot however be used for baseline work in Nigerian cattle because all the sampled cattle should be negative for helminthosis absoluately. Other scientific techniques are required to validate these values in further studies.
6. Recommendations

The use of measurement of Acute Phase Proteins (APPs) on a herd basis is very important for maximum monitoring of health status of cattle and to prevent the occurrence of helminthicosis in cattle ranches. Since APPs can be isolated in milk (which is cheaper), large scale measurement of these proteins can be achieved. There is a strong need for this same study to be carried out in different breeds of small domestic ruminants in Nigeria as these species of animals are more prone to helminthicosis. Nigerian cattle farmers need to be educated on the need to regularly deworm their stock considering the acute phase proteins response recorded even at very low level of worm burden. Also this same study can also focus on other classes of helminths such as members of class Trematoda (liver flukes) and class Cestoda (tape worms).

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References


