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Evaluation of Serum Amyloid A and Haptoglobin in Dairy Cows Naturally Infected with Brucellosis

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Abstract

Brucellosis is one of the most important zoonotic diseases worldwide, which is responsible for a debilitating disease in humans and a chronic infection in domestic animals. The aim of this study was to determine serum changes of two major acute phase proteins (Serum Amyloid A (SAA) and Haptoglobin (Hp)) levels in dairy cows naturally infected with brucellosis. The study included 25 dairy cows with brucellosis and 25 healthy cows. It was found that mean SAA levels (μg/ml) were significantly ($P < 0.05$) higher in brucellosis cases (123.75 ± 12.64) as compared to values measured in the control group (32.92 ± 9.12). In addition, SAA levels measured in the positive cases correlated with the increase in antibody levels, both in 2-mercaptoethanol (2ME) and Wright tests. However, the analyses of the results between the evaluated groups did not show any significant differences in measured serum concentration of Hp (g/l) ($P > 0.05$). Our results suggested that some acute phase proteins are involved in the pathophysiology of brucellosis and are closely related to the inflammatory activation of the disease. In view of the present findings, it is suggested SAA may be used as an indicator for bovine brucellosis.

Keywords: Brucellosis; Acute phase proteins; Serum Amyloid A; Haptoglobin; Dairy cows

Introduction

Over the last few years, Acute Phase Proteins (APPs) have become the biomarkers of inflammation and infection for diagnostic and prognostic purposes in both farm and companion animals [1,2]. APPs are produced in response to a variety of disease conditions stimulated by the pro-inflammatory cytokines and in response to infection, inflammation, surgical trauma or stress. The APPs consist of 'negative' and 'positive' proteins that show a decrease and an increase in concentration, respectively, in response to challenge [1,3,4]. The wide nature of the APP response can be seen as a disadvantage in that APP assays are not specific for one disease, but are shared with other long-established diagnostic tests in the clinical repertoire [5]. On the other hand, various infections and inflammatory processes may be associated with different patterns in acute phase reactivity [6]. More detailed knowledge of the patterns of response of different APPs may allow us to use them more effectively in diagnosis and prognosis [3].

In cattle, elevated blood serum concentrations of haptoglobin and SAA, as two major acute phase proteins, have been demonstrated in association with several diseases [1-3,7]. However, to the best of our knowledge, there are limited published reports describing the influence of *Brucella* infection on the concentrations of acute phase proteins (SAA and Hp) in dairy cows. More recently, a comparative proteomic study on serum of brucellosis dairy cows suggested SAA as a biomarker of brucellosis-associated protein [8].

Brucellosis is a major bacterial zoonosis with significant economical and zoonotic impact. The causative organisms are gram-negative facultative intracellular pathogens that may affect a range of different mammals including man, cattle, sheep, goats, swine, rodents and marine mammals. In most host species, the disease primarily affects the reproductive system with concomitant loss in productivity of the animals affected [9]. Eradication of brucellosis has been a goal for many countries, with success in several countries in northern Europe. Those countries that do eradicate infection cannot afford to be complacent as the threat of reintroduction is ever present through the movement of livestock. In order to control brucellosis, comprehensive surveillance, pre and post-import testing is of paramount importance [9,10].

In recent years remarkable progress has been made towards understanding the biology of the *Brucella*, however, our comprehension of pathogenicity mechanisms remains evasive. Results from acute phase proteins investigation in cows would eventually lead to a greater understanding of the mechanisms employed for the highly adapted pathogenesis of these microbes.

In the present study we hypothesized that brucellosis in cows may affect the concentrations of major acute phase proteins.

Materials and Methods

Animals and experiment

The study was performed between March and April 2010. Blood samples were obtained by venipuncture and were transported in ice-boxes to the laboratory, as soon as possible. The serum was then separated by centrifuging the blood samples at 2000 g and kept at -20°C until tested. Serum antibodies were investigated for the presence of antibodies against *Brucella* genus using slide agglutination by Rose Bengal Plate Test (RBPT) and tube agglutination test by Wright and 2-mercaptoethanol (2ME), using whole cell antigen (Razi Vaccine and Serum Research Institute) [11]. Positive serology was defined as Wright titer ≥ 1/160 plus 2ME ≥ 1/80. Control group was composed of healthy blood donors with no history of brucellosis. Cows were divided

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into two groups (positive=25 and negative=25 cases) according to their serological results (RBPT, Wright and 2ME). The control group consisted of clinically healthy cows of the same age range, nutrition and breed. The influence of natural bovine brucellosis on the concentrations of selected acute phase proteins (Hp and SAA) was investigated in infected dairy cows compared to healthy cows.

Acute phase proteins (Hp and SAA) determination

Hp was measured according to 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). SAA was 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).

Statistical analysis

Descriptive statistics were calculated as means, medians and standard deviations. Due to significant deviation of the data from normality ($P < 0.05$), assessed by the Kolmogorov–Smirnov test, comparison between means was performed using a non-parametric Kruskal–Wallis test. To estimate the association between study variables, Pearson's correlation coefficient and Spearman's rank correlation were calculated.

Results

Statistical analysis was performed to assess the pattern of changes and the relative value of APPs (including Hp and SAA) and to find a possible relationship between antibody titers and APPs changes in bovine brucellosis.

The average SAA concentration was significantly higher ($P<0.05$) in cows positive for brucellosis as compared with healthy animals (Table 1). However, the analyses of results between the evaluated groups showed no significant differences in measured serum concentration of Hp ($P > 0.05$). The data referring to the concentrations of evaluated acute phase proteins in healthy animals and cows with brucellosis are presented in table 1.

As the level of antibodies increased in serum of infected cattle in Wright and 2ME tests, a significant increase was observed in SAA ($r=0.37$, $P<0.05$ and $r=0.33$, $P<0.05$, respectively). In contrast, no significant increase in Hp was observed as antibody titers and SAA increased (Table 2).

Discussion

The APPs have been investigated as biomarkers of disease in ruminants and particularly in cattle for a number of decades [12]. Applications of 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. The other objective of the investigation of the APP is to understand the pathophysiology of disease processes

Acute phase proteins	Group of cows		P
	Healthy (Brucella negative) (n=25)	Brucella positive (n=25)	
Hp (g/l)	0.61 ± 0.08	0.62 ± 0.09	P ≥ 0.05
SAA (µg/ml)	32.92 ± 9.12	123.75 ± 12.64	P ≤ 0.05

Table 1: The mean ± SD for Hp (g/l) and SAA (µg/ml) concentration in the two groups of infected and healthy dairy cows.

	Hp (g/l)	SAA (µg/ml)	Wright	2ME
Hp (g/l)	1.00	-	-	-
SAA (µg/ml)	-0.06	1.00	-	
Wright	-0.26	0.37*	-	-
2ME	-0.29	0.33*	0.92**	1.00

* P ≤ 0.05
** P ≤ 0.01

Table 2: The summary statistics for correlations among Hp (g/l), SAA (µg/ml), Wright and 2ME antibody levels.

involved in the innate immune response to infections. 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 [2,12]. Diagnostic methods for brucellosis have primarily been based on serology with the LPS from smooth strains producing the greatest immunological responses in various hosts. A major limitation of serological assay in its application as a routine diagnostic test is its low sensitivity for detection of antibody, particularly during the early phase of disease, whereas the maximum serum concentration of APPs is typically reached within 24 to 48 h after the initiation [13,14].

The magnitude and duration of the acute phase response reflect the severity of the infection and underlying tissue damage [15]. Among different APPs, 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 [3,6].

In our study, the presented results in cows with brucellosis showed a markedly higher concentration of SAA than in healthy cows, but in the concentrations of Hp no marked differences between these two groups of cows were found. These findings might be a consequence of a different initiation of the production of various acute phase proteins, seeing that SAA is a more sensitive acute phase protein than Hp in cattle, with rapid increase in serum concentrations after the inflammatory stimulus [16]. There are also indications that the response to chronic compared to acute inflammation varies from one protein to another [14].

Haptoglobin (Hp) is a glycoprotein composed of 2α and 2β subunits. The primary function of Hp is to bind free hemoglobin in the blood [17]. By removing any free hemoglobin which has inherent peroxidase activity from the circulation, Hp prevents oxidative damage of tissues [18]. Many studies have indicated the significance of Hp as a clinically useful parameter for measuring the occurrence and severity of inflammatory responses in cattle with mastitis, pneumonia, enteritis, peritonitis, endocarditis, abscesses, endometritis and other natural or experimental infectious situations [19,4]. Haptoglobin is used to monitor the treatment efficacy of antibiotics in cows with toxic puerperal metritis [20]. Hp is also used to determine the effect of anti-inflammatory drugs following the castration of bull calves, the relative effects of bacterial contamination and involution of the uterus in dairy cows after calving, the effects of treatment in transport-stressed feedlot cattle, the effects of tail docking or surgical castration, and the changes in the blood profile of neonatal calves [21]. Haptoglobin is also induced in cows with fatty liver syndrome, by starvation, and in calves following stress associated with road transport [22].

In human Brucellosis, some researchers previously showed that the CRP in the patients affected by brucellosis (25 ± 20.7) is obviously increased in comparison with healthy persons (6.9 ± 4.4) [23]. The

same authors could not show any correlation between level of serum CRP and serological results in patients. In contrast to this, we recorded a significant correlation between the concentrations of SAA in the serum and antibody levels in Wright and 2ME tests.

Kujala et al. [24] showed higher mean concentration of SAA in lame cows due to sole ulcer and white line disease than in healthy animals. However, in Hp concentrations they found no significant differences between healthy and lame cows. Therefore, the authors suggested that SAA is a better indicator for claw disorders than haptoglobin. Werling et al. also reported that SAA is a more sensitive acute phase protein than Hp in cattle with rapid increase in serum concentrations after the inflammatory stimulus. According to Muller-Doblies et al. [25], Hp requires a stronger stimulation to induce an increase in serum concentrations.

Recent advances have shown that Brucella infection elicits only moderate inflammatory responses, which is likely the result of strategies both to "hide" from immune detection and to actively suppress generation of host immune responses. However, some questions about the biology of *Brucella* spp. remain to be answered. In dairy cows, the disease occurs as a chronic infection that results in placentitis and abortion in pregnant cases [26]. *B. abortus* induced abortion is associated with necro-hemorrhagic placentitis and fetal lesions, particularly fibrinous pleuritis and pericarditis, and interstitial pneumonia [27]. Accordingly, the production of APP is stimulated in liver following the release of cytokines such as IL-1, IL-6, and TNF-α from macrophages and monocytes at the site of inflammatory lesions or infections [14,28].

Serum amyloid A (SAA) is a small hydrophobic protein (9–14 kDa), which is found in serum associated with high density lipoprotein [29]. Recently, a direct antibacterial action of SAA was identified, in which SAA was found to bind to Gram negative bacteria leading to opsonisation of the target pathogen [30]. The antibacterial activity of bovine SAA is probably wider, since it is directed toward both Gram+ and Gram– bacteria [31].

There are, however, inconsistent results about changes in SAA and Hp concentrations and their correlation in various inflammatory conditions. Some studies have indicated that the pattern and magnitude of SAA and Hp do not differ significantly and their value as indicators of disease is equal [32,33], while others have reported that SAA and Hp change rather differently [34-36]. This suggests that various infections and inflammatory processes may be associated with different patterns in acute phase reactivity [6] and that a more detailed knowledge of these patterns may be useful in establishing a differential diagnosis [3].

Future studies would likely examine how acute phase proteins expressed within host cells aid pathogen survival and/or induce host responses. Experimental researches are needed to determine the best choice of acute phase proteins and to show stage-dependent pattern of their alterations in serum over the course of infection.

Conclusions

In conclusion, measuring SAA with the higher sensitivity compared to the Hp can be a suitable biochemical marker of inflammatory reactions in dairy cows naturally infected with Brucellosis.

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