Haptoglobin and Serum Amyloid A Responses in Cattle Persistently Infected with Bovine Viral Diarrhea Virus

Bulent Ulutas¹, Tolga Tan², Pinar Alkim Ulutas³ & Goksel Bayramli¹

ABSTRACT

Background: The acute-phase response to infection is also characterized by an increase in protein turnover plus an increased loss of protein. Leukocyte proliferation and the synthesis of cytokines, immunoglobulins and positive acute phase proteins (APPs) contribute to protein turnover. APPs levels, as markers of inflammation, usually rise significantly during the course of acute and chronic infections in the bovine. There has been a great interest shown on the usage of APPs as a marker of animal health or, alternatively, as an indicator of disease severity in veterinary medicine. Haptoglobin (Hp) and serum amyloid A (SAA) are important APPs in cattle, in different viral and bacterial infections. The purpose of the present study was to investigate the influence of infection on acute-phase proteins in seldom persistent positive BVDV cattle, that were free of signs and symptoms of secondary infections.

Materials, Methods & Results: Blood samples, from a total of 313 cows from three different farms, were collected in 10 mL polystyrene tubes coated with an anticoagulant (EDTA). Commercial BVDV antigen ELISA kits were used to determine the persistently infected animals. Sampling was carried out by two occasions within 40 days intervals. A total of 11 dairy cows were found BVDV Ag positive in both occasions, were evaluated as persistently infected animals. Sampling was carried out within 40 days intervals. A total of 11 dairy cows were found BVDV Ag positive in both occasions, were evaluated as persistently infected. Eight non-infected dairy cows from same herds were served as control group. All animals were examined on the day of blood sampling. The levels of haptoglobin and SAA in serum were determined by use of the commercial kits. Statistical analyses were performed by Mann-Whitney U-test; \( P < 0.05 \) considered significant. None of the persistently infected animals showed clinical findings and a few of them were considered as poor doers, but they were unnoticed until they were identified as persistently infected animals. No clinical findings were observed in healthy control cows in routine clinical examination. Serum concentration of Hp in persistently infected cattle with BVDV was significantly \( (P < 0.05) \) higher than the controls. Serum amyloid A concentrations were also significantly higher in persistently infected cattle with BVDV than the controls.

Discussion: APPs have been proposed as valuable indicators of the manifestation and severity of pathological conditions in humans and animals. Levels of APPs are preferentially elevated during acute bacterial infections and less pronounced or even missed during viral infections. There are no reports on the alterations of the acute-phase protein profile in the persistent BVDV infection. In the present study, a significant increase in the positive acute-phase proteins haptoglobin and SAA in the symptom-free persistent cattle were detected. These alterations of acute-phase proteins may be associated to the enhanced production of IL-6 and IL-8 which modulate the synthesis of positive acute-phase proteins. Clinical symptoms were not noticed in our study. Such animals could easily be underdiagnosed in a farm population. Clinical symptoms were associated with increase in APPs. Moreover, supra-normal APP values in persistently infected cattle with BVDV were observed without clinical symptoms. The role of inflammation as defined by Hp and SAA levels have not been fully explored in the context of persistently infected cattle with BVDV disease. The results of this study indicated that serum concentrations of haptoglobin and SAA were increased in persistently infected cattle. Moreover, it may be suggested that measurement of SAA and Hp may be of valuable in indicating suspected persistently infected animals with BVDV in screening herds for general health status.

Keywords: persistent BVDV, cattle, serum amyloid A, haptoglobin.
INTRODUCTION

The acute-phase response to infection is also characterized by an increase in protein turnover plus an increased loss of protein [23]. Leukocyte proliferation and the synthesis of cytokines, immunoglobulins and positive acute-phase proteins (APPs) contribute to protein turnover [14]. APPs levels, as markers of inflammation, usually rise significantly during the course of acute and chronic infections in the bovine. There has been a great interest shown on the usage of APPs as a marker of animal health or, alternatively, as an indicator of disease severity in veterinary medicine [12,21].

Haptoglobin (Hp) and serum amyloid A (SAA) are important APPs in cattle [2,18], in different viral [15,20] and bacterial infections [9,21]. Godson et al. [16] reported on the sequential infection of cattle with bovine herpesvirus 1 and Pasteurella multocida and the concomitant rise of Hp, which was prompt and distinct after the secondary bacterial infection. At the same time, it was recognized that the maximum level of Hp in serum was a valuable prognostic factor and reflects the severity of the disease.

SAA is one of the acute phase protein, most sensitive to viral infections in man [30]. Experimental bovine respiratory syncytial virus infection induced strong SAA and haptoglobin acute phase responses in cattle [20]. In other studies relevant to viral infections, such as experimental bovine herpes virus infection [16] or naturally acquired rinderpest virus [28], no elevations were detected on haptoglobin levels. Indeed haptoglobin response was reported in foot-and-mouth disease virus infections [21].

The clinical signs in persistently infected animals that have not (yet) developed mucosal disease, may encompass a wide spectrum of clinical signs, ranging from normal health to subclinical disorders, ill-thrift and growth retardation. Chronic or recurrent intestinal and/or pulmonary symptoms are frequently observed but occasionally dermatological, neurological or haematological disorders may be the reflections of a persistent infection [3,7,29].

Reports of increased protein turnover in humans with symptom-free Acquired Immunodeficiency Syndrome suggest that the infection by the virus, in the absence of clinical signs and symptoms, can induce changes in protein metabolism [23]. However to the present authors’ knowledge, there is scarcity information regarding the alterations of the acute-phase protein profile in the persistent Bovine Viral Diarrhea Virus (BVDV) infection in cattle.

Therefore the purpose of the present study was to investigate the influence of infection on acute-phase proteins in seldom persistent positive BVDV, that were free of signs and symptoms of secondary infections.

MATERIALS AND METHODS

Virological Studies

Blood samples, from a total of 313 cows from three different farms, were collected in 10 mL polystyrene tubes coated with an anticoagulant (EDTA). Commercial BVDV antigen ELISA kits were used to determine the persistently infected animals. Whole blood samples were used and the test was performed according to the manufacturer’s instructions. Sampling was carried out by two occasions within 40 days intervals. A total of 11 dairy cows were found BVDV Ag positive in both occasions, were evaluated as persistently infected.

Eight non-infected dairy cows from same herds were served as control group. All animals were examined on the day of blood sampling. All animals in two groups were examined for the clinical signs of illness such as fever, inappetence, nasal discharge, abnormal feces etc.

Acute Phase Proteins

The levels of Hp and SAA in serum were determined by use of the commercial kits Haptoglobin level in serum was determined by the haemoglobin binding method using micro-titre plates and SAA was measured by sandwich ELISA using phase SAA kits in ELISA reader according to the manufacturer’s instructions.

Statistical analyses were performed by Mann-Whitney U-test with SPSS software \(P < 0.05\) considered significant.

RESULTS

None of the persistently infected animals showed clinical findings and a few of them were considered as poor doers, but they were unnoticed until they were identified as persistently infected animals. No clinical findings were observed in healthy control cows in routine clinical examination.
The results were shown in Table 1. Serum concentration of Hp (0.357 ± 0.59 mg/mL) in persistently infected cattle with BVDV was significantly \((P < 0.05)\) higher than the controls (0.176 ± 0.03 mg/mL). Serum amyloid A concentrations (49.98 ± 14.64 \(\mu\)g/mL) were also significantly \((P < 0.05)\) higher in persistently infected cattle with BVDV than the controls (13.64 ± 4.6 \(\mu\)g/mL).

### Table 1. Serum concentrations of Hp and SAA in healthy and persistently infected cattle with BVDV.

<table>
<thead>
<tr>
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<th>Haptoglobin (mg/mL) Mean±SE</th>
<th>Serum Amyloid A ((\mu)g/mL) Mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistently Infected (n=11)</td>
<td>0.357 ± 0.59*</td>
<td>49.98 ± 14.64*</td>
</tr>
<tr>
<td>Healthy (n=8)</td>
<td>0.176 ± 0.03</td>
<td>13.64 ± 4.6</td>
</tr>
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\* \(P < 0.005\)

### DISCUSSION

APPs have been proposed as valuable indicators of the manifestation and severity of pathological conditions in humans and animals. Levels of APPs are preferentially elevated during acute bacterial infections, notably during bovine mastitis [13] and less pronounced or even missed during viral infections [4,18,19,24]. Godson et al. [16] reported on the sequential infection of cattle with bovine herpesvirus 1 and Pasteurella multocida and the concomitant rise of Hp, which was prompt and distinct after the secondary bacterial infection. At the same time, it was recognized that the maximum level of Hp in serum was a valuable prognostic factor and a mirror of the disease severity. The purpose of the present study was to investigate the effect of infection by persistent BVDV alone on acute-phase proteins in positive persistent BVDV cattle including those free of signs and symptoms of secondary infections. There are no reports on the alterations of the acute-phase protein profile in the persistent BVDV infection. In the present study, a significant increase in the positive acute-phase proteins Hp and SAA in the symptom-free persistently infected cattle were detected. These alterations of acute-phase proteins may be associated to the enhanced production of IL-6 and IL-8 which modulate the synthesis of positive acute-phase proteins [1,31].

Acute phase proteins have been proposed as valuable indicators of the manifestation and severity of pathological conditions in humans and animals. In cattle, SAA and Hp are recognized as major APPs but their responses vary in individual animals. Varying magnitude of the acute phase response in different viral [20,21] and bacterial infections [9,15] has been shown. APP levels may be elevated during acute bacterial infections, notably during bovine mastitis [10,13] and less pronounced or even may be missed during the course of viral infections [4,18,19,24].

Genheim et al. [15] have reported that the serum Hp concentrations were between 0.89 - 1.77 mg/mL and SAA concentrations were between 77.7 - 375 \(\mu\)g/mL in cattle experimentally infected with transient BVDV, respectively. The critical aspect of the study was the evaluation of the acute phase protein response in persistently infected cattle with BVDV. In our study, mean serum Hp and SAA concentrations in cattle persistently infected with BVDV was significantly \((P < 0.05)\) higher than the controls. Furthermore, the magnitude of the response was lower than the response in cattle infected with BVDV and/or Mannheimia haemolytica as reported before by Genheim et al. [15]. However, clinical symptoms were not noticed in our study. Such animals could easily be underdiagnosed in a farm population. Clinical symptoms were associated with increase in APPs. Moreover, supra-normal APP values in persistently infected cattle with BVDV were observed without clinical symptoms. In our study, these changes in Hp and SAA values in persistently infected cattle with BVDV may be useful in an attempt to identify animals that are or have recently been, clinically or subclinically diseased to a chronic inflammation as described before by Horadagoda et al. [22]. Therefore such values may be useful as a diagnostic tool when screening herds for subclinical or clinical disease to evaluate animal health.
Although the APP response to bacterial infections has been well documented and established in the veterinary literature, very few studies have investigated the response to viral infections. Typically APP response to the stress of a bacterial infection, positive APPs concentrations increase [5,8,23,25] and there might be a two- to fourfold increase in response to injury or infection [6,11,14,24,27]. However it is not very well known whether this APP response can be elicited by chronic subclinical viral infections. Limited data exists in the literature suggesting that, whereas acute viral infections would elicit an APP response, chronic subclinical viral infections would not. Higher plasma concentrations of positive APPs including C reactive protein (CRP) and SAA were detected in children infected with measles, varicella, rubella, and echo-30 meningitis but not in children with chronic hepatitis B and C [26]. It has been reported that HIV-infection promotes an enhancement of the fractional and absolute synthesis rates of positive acute-phase proteins, including Hp [23]. Previously reported data on acute-phase proteins in HIV infection are conflicting [17] reported higher plasma concentrations of C reactive protein in a group of AIDS patients compared to the plasma concentrations of healthy controls; however, no difference in the plasma concentration of haptoglobin between the 2 groups was found. Jahoor, et al. [23], found that the symptom-free AIDS group had significantly higher plasma C reactive protein, fibrinogen and haptoglobin concentrations than the control group. The disagreement between these studies may be attributed to the different stages of HIV-infection of the AIDS patients broadly classified as symptom-free in each study, as well as to the inter-individual variation of the immune response among these patients. According to our data, it may be concluded that the persistent infected BVDV-induced acute phase response, and a lesser magnitude than that observed in response to a bacterial infection [23].

The role of inflammation as defined by Hp and SAA levels have not been fully explored in the context of persistently infected cattle with BVDV disease. The results of this study indicated that serum concentrations of Hp and SAA were increased in persistently infected cattle. Moreover, it may be suggested that measurement of SAA and Hp may be of valuable in indicating suspected persistently infected animals with BVDV in screening herds for general health status.

SOURCES AND MANUFACTURERS
1 Bio-X Diagnostics Belgium, Belgium.
2 Tridelta Ltd, Kildare, Ireland.
3 Anthos2010, Eugendorf, Austria.

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

REFERENCES


