SERUM HAPTOGLOBIN AND AMYLOID A CONCENTRATIONS AND CLINICAL FINDINGS IN SHEEP WITH PESTE DES PETITS RUMINANTS

HANDAN HILAL ARSLAN, SENA CENESIZ¹, CEVAT NISBET¹, AND ZAFER YAZICI²

Department of Internal Medicine, ¹Department of Biochemistry, ²Department of Virology, Faculty of Veterinary Medicine, University of Ondokuz Mayis, 55139 Samsun, Turkey
hharslan@omu.edu.tr

Received for publication April 10, 2007

Abstract

The changes in serum haptoglobin (Hp) and amyloid A (SAA) concentrations in sheep with peste des petits ruminants (PPR) were investigated. Additionally, the relationships between these acute phase proteins and clinical symptoms were studied. Twenty sheep showing PPR virus antibodies in c-ELISA and 10 sheep PPR virus negative were used as the experimental and control groups. In the clinical examination, typical clinical symptoms, such as fever, nasal catarrh, erosive stomatitis, gastroenteritis, and conjunctivitis were detected in all PPR affected animals. In addition, statistically significant differences were found in body temperature and respiratory and heart rates between experimental and control groups (P<0.001). SAA and Hp levels increased significantly (P <0.001) in PPR virus infected sheep in comparison with healthy animals. Consequently, the obtained data showed that although the acute phase proteins were not specific for the disease, serum Hp and SAA could be used as an indicator together with clinical findings in sheep with PPR.

Key words: sheep, peste des petits ruminants, symptoms, haptoglobin, amyloid A.

Peste des petits ruminants (PPR) is an important viral disease of goats and sheep that affects the economics of farming the animals (10). The disease was first described in West Africa in the 1940s (5, 6). It is included in the list A of the International Zoosanitary Code, and is a part of the FAO EMPRESS programme (7). PPR virus (PPRV) occurs in Africa, Middle East, Arabian Peninsula, and Southern Asia (5, 10, 12). PPRV infection was recently officially reported in Turkey, in 1999 (12).

PPRV is a morbillivirus and is closely related to rinderpest virus (RPV), canine distemper virus, and the human measles virus (5, 12). Like other morbillivirus infections, PPRV spreads by close contact between infected and susceptible animals (12). The infection with PPRV results in an acute, highly contagious disease characterised by fever, anorexia, necrotic stomatitis, diarrhoea, purulent ocular and nasal discharges, and respiratory distress. The death ratio is usually 40-80% of acute cases (7). The virus is rapidly inactivated in dead animals (4). Animals that recover do not become carriers (12, 18)

Three clinical forms appear in animals with PPR. Sheep and, less commonly, goats develop subacute reactions after an incubation period of about 6 d. In 10-14 d the animals usually recover. The second form of the disease is the acute reaction. This form begins 3-4 d after an incubation period. Fever and serous rhinorrhoea suddenly appear, but otherwise the affected animals look normal. The third form is the peracute reaction that follows incubation period just after 2 d. A profuse nasal catarrh precedes a sudden high fever with signs of depression, dyspnoea, anorexia, and constipation (17).

The body’s early defence is a complex set of systemic reactions to tissue injury, neoplastic growth, immunologic disorders, inflammation or infection, seen shortly after the exposure to a triggering event. One of the components is an acute phase protein response in which serum concentration of positive acute phase proteins increases. The serum concentration of these proteins returns to basis levels when the triggering factor is no longer present (13, 22). In cattle, the most sensitive acute phase proteins are serum haptoglobin (Hp) and serum amyloid A (SAA), and concentrations of these proteins increase particularly in the response to acute inflammatory conditions as well as to subclinical inflammation (23). The determination of the SAA can be of value to the veterinarian in helping to identify animals with inflammatory diseases (9).

Hp is useful as a marker for the presence of bacterial infection in sheep, and is more sensitive, specific, and efficient and less likely to give false positive and negative results than haematological examinations (19). The SAA protein family are one of the major reactants in the acute-phase response (11). In some studies it was demonstrated that both Hp and SAA
had a low sensitivity but higher specificity in determining disease status, compared with clinical examinations of cattle (8).

In this study, the significance of acute phase proteins Hp and SAA for the detection of the systemic inflammatory response in sheep affected with PPR was determined. Additionally, the relationship between acute phase proteins and clinical symptoms in these sheep was investigated.

**Material and Methods**

**Animals and clinical examinations.** The study was performed on 20 sheep showing clinical symptoms of PPR (experimental group) and 10 healthy sheep (control group). Body temperature, and respiratory and heart rates were measured and statistically evaluated. The sheep neither had PPR infection before, nor were vaccinated against PPR. Competitive enzyme linked immunosorbent assay (c ELISA) was used for serological detection of PPRV specific antibodies. All procedures were carried out according to the Manual of Standards issued by the Office International des Epizooties (3).

**Determination of acute phase proteins.** Acute phase proteins were determined and evaluated in PPR positive and control animals. For this aim, blood samples were collected from the jugular vein into empty tubes. Serum was separated from clothing blood by centrifugation of the tubes at 1 550 g for 10 min and the serum samples were stored at –70 °C until the analysis. Serum concentrations of Hp and SAA were detected with commercially available kits (Tridelta Development Limited, Ireland) according to the manufacturer’s instruction. A Digital and Analogue Systems (Italy) ELISA microplate reader was used.

**Data evaluation.** Mann-Whitney U test for two independent samples was used for statistical comparison between experiment and control groups (16).

**Results**

During clinical examinations, typical clinical symptoms, such as fever, catarrh, erosive stomatitis, gastroenteritis, and conjunctivitis were detected in all PPR suspected animals. No clinical symptoms were observed in the control group animals. In addition, statistically significant differences were found in body temperature and respiratory and heart rates, between the control and PPRV positive animals (P<0.001) (Table 1). PPRV antibodies were detected in all serum samples of suspected animals.

A significant increase in the mean serum Hp and SAA concentrations of infected sheep in comparison with healthy sheep (P<0.001) was demonstrated. In some sick animals, serum Hp and SAA levels were found about two times higher than those in the control group. In addition, SAA concentrations in some cases were six times higher than those in control group. Serum Hp and SAA concentrations are shown in Table 2.

**Discussion**

PPR is an acute and highly contagious viral disease of small ruminants, such as sheep and goats, with high morbidity and mortality rates (10). PPR infection is still a major problem causing sporadic outbreaks among sheep, goats, and wild small ruminants. It also causes great economic losses and poses threats to national livestock industries, especially in sub-Saharan Africa and Middle East (3, 4, 18).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Body temperature (X ± Sx)</th>
<th>Respiratory rate (X ± Sx)</th>
<th>Heart rate (X ± Sx)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>10</td>
<td>38.47 ± 0.70</td>
<td>26.50 ± 5.23</td>
<td>94.00 ± 7.79</td>
</tr>
<tr>
<td>Experimental group</td>
<td>20</td>
<td>40.10 ± 0.70*</td>
<td>41.25 ± 2.36*</td>
<td>117.15 ± 2.94*</td>
</tr>
</tbody>
</table>

* P<0.001 as compared to controls.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Hp (X ± Sx)</th>
<th>SAA (X ± Sx)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>10</td>
<td>1.54 ± 0.20</td>
<td>12.80 ± 4.15</td>
</tr>
<tr>
<td>Experimental group</td>
<td>20</td>
<td>3.13 ± 0.94*</td>
<td>32.3 ± 18.48*</td>
</tr>
</tbody>
</table>

* P<0.001 as compared to controls.
Clinical symptoms of PPR in domestic and wild small ruminants are fever, erosive, necrotic stomatitis, purulent ocular and nasal discharges, conjunctivitis, gastroenteritis, and pneumonia (1, 12, 17). In the present study, the clinical findings were compatible with previous reports. Fever, nasal catarrh, erosive stomatitis, gastroenteritis, and conjunctivitis were detected in all the affected animals. Body temperature, respiration rate, and heart rate were statistically significantly different between the control and PPRV positive groups (P < 0.001).

The acute phase response in serum is a well-recognized indicator of inflammation, infection, trauma, and other pathological injuries (22, 23). In cattle, the most sensitive acute phase proteins are Hp and SAA, and their concentrations increase particularly in response to experimental or natural acute or subclinical inflammation (22). In sheep, the measurement of the concentrations of ceruloplasmin, fibrinogen, and Hp may be more useful in the diagnosis of tissue injury and infectious disease than the number of circulating neutrophils (14). Pfeffer et al. (15) also reported that the "positive" acute phase proteins may be useful indicators of production losses due to inflammatory diseases in sheep.

Skinner and Roberts (19) reported that Hp was useful as a marker for the presence of bacterial infection in sheep, and was more sensitive, specific, and efficient and less likely to give false positive and negative results than a haematological examinations. The SAA response during viral respiratory diseases was well described. As for Hp, the SAA response is of longer duration in mixed viral and bacterial infections when compared to pure viral infections (13).

Furthermore, the combined measurement of two or three acute phase proteins should be used to achieve the highest sensitivity for the detection of ongoing infection during a prolonged time period (21). In some cases, both Hp and SAA had a low sensitivity but higher specificity in determining disease status compared with clinical examination (8). A diagnostic tool based on such acute phase protein measurements could considerably improve strategic procedures for control of the infection (8, 21). The plasma concentrations of SAA and Hp and the Hp/SAA ratio are useful parameters to distinguish healthy animals from animals with inflammation and can be helpful in distinguishing between acute and chronic inflammatory diseases (2). In this study, SAA and Hp levels were significantly increased in PPR infected animals, which showed clinical signs of the disease.

Early diagnosis and prognosis evaluation are important for PPR control. In this sense, like other bacterial and viral disease, acute phase protein levels are not suitable for establishing a specific diagnosis but they can provide objective information about the extent of ongoing lesions in individual animals with PPR. At a herd level, acute phase proteins might be useful for determining where the spread of the disease is taking place (age group, part of the production system), by providing information about the prevalence of ongoing clinical and subclinical infections indicated by the high serum concentration of selected acute phase proteins (13). It may serve as a prognostic tool, with the magnitude and duration of the acute phase response reflecting the severity of infection (13, 20).

In this study, SAA and serum Hp concentrations were found to be significantly increased in naturally PPRV infected sheep. In addition, clinical symptoms were compatible with results of laboratory analysis. Consequently, the obtained data showed that acute phase proteins could be used as an indicator and supplementary laboratory test together with laboratory and clinical findings in sheep with PPR. However, acute phase response parameters used in the study could be treated only as an orientative indicator, poorly indicative of an individual kind of infection, which requires subsequent microbiological confirmatory investigations. We could not find another report about the relationship between PPR and acute phase proteins in sheep. Therefore, more studies should be performed in such cases.

References


