

# Haptoglobin in goats with caprine arthritis-encephalitis

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## Abstract

Concentration of haptoglobin (Hp) was measured in 325 goat serum samples using guaiacol (peroxidase) test. One hundred forty six samples (45%) originated from goats infected with small ruminant lentivirus (SRLV), etiological agent of caprine arthritis-encephalitis (CAE), whereas 179 (55%) from non-infected goats. Mann-Whitney U test was applied to evaluate statistical significance of a difference between Hp concentrations in CAE-positive and CAE-negative sera. Totally, 11 serum samples were positive for Hp – 4 CAE-positive (2.7%) and 7 CAE-negative (3.9%). Hp concentrations in 4 CAE-positive samples were 0.17, 0.83, 1.28, 9.25 g/l, whereas Hp concentrations in remaining 7 CAE-negative serum samples were 0.11, 0.16, 0.19, 0.49, 0.62, 0.91 and 1.21 g/l. No statistically significant difference between Hp concentrations in CAE-positive and CAE-negative sera could be found. However median of Hp concentrations in goats with detectable levels of Hp in the former group (1.06 g/l) was more than 2-fold higher than in the latter group (0.49 g/l).

**Key words:** acute phase proteins (APP), caprine arthritis-encephalitis (CAE), haptoglobin (Hp), small ruminant lentivirus (SRLV).

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## Introduction

Haptoglobin (Hp) is a plasma  $\alpha_2$ -glycoprotein, produced in the liver, which scavenges free hemoglobin and transports it to the hepatocytes, participating in recovering iron from eliminated erythrocytes [1]. Moreover, haptoglobin, together with many other proteins such as C-reactive protein, serum amyloid A, fibrynogen and  $\alpha_1$  acid glycoprotein, form a group of acute phase proteins (APP). They are proteins whose concentration changes in the response to the damaging factors, being a part of the inflammatory reaction. Their concentration rises in many infectious diseases in humans and animals, including goats [2, 3]. Hp is deemed a major positive acute phase protein in ruminants [4] as its concentration rises more than 10 times in response to the stimulation [5]. It is induced by interleukin 6, secreted by monocytes, and it exerts bacteriostatic effect due to restriction of the availability of free iron [6].

To date, Hp concentration has been assessed in several different diseases in small ruminants. They were acute metabolic conditions like pregnancy toxemia in goats [7] but also infectious and parasitic conditions such as ovine caseous lymphadenitis [8], mixed helminth infection [9] and sarcoptic mange [10]. Whereas Hp is proven to rise considerably in acute conditions and remain elevated for up to 2 weeks [5, 11, 12], its behavior in chronic infectious diseases in ruminants is still unclear.

Caprine arthritis-encephalitis (CAE) is a chronic infectious disease of goats caused by the lentivirus (family *Retroviridae*). This virus had long been called caprine arthritis-encephalitis virus (CAEV) and considered distinct from the virus responsible for Maedi-Visna disease but recently they have been reclassified into one species called Small Ruminant Lentiviruses (SRLV) [13]. They infect monocytes and macrophages, leading to their permanent stimulation and impaired secretion of inflammatory mediators [14]. It results

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in chronic immune-mediated inflammation of the tissues, which harbor infected macrophages. Joints are most commonly affected in goats but the disease may also involve lungs and udder or manifest itself as a chronic wasting [15]. As the development of lesions is slow and insidious, clinical signs are observed in adult goats. However, the virus is also capable of damaging central nervous system in kids leading to progressing irreversible paralysis of the limbs [16].

The objective of the study was to check if there is any relationship between infection with SRLV and concentration of Hp in goats.

## Material and methods

Serum samples from 325 goats were selected to the study from the samples collected for routine diagnosis of CAE. The CAE-positive samples originated from 10 herds in which SRLV infection was confirmed in two serological surveys 5 years apart and CAE-negative samples originated from 7 herds classed in the same manner as free from SRLV infection. The serum samples were tested for antibodies against SRLV with ELISA test (IDEXX CAEV/MVV Total Ab Screening Test). The test was performed according to the manufacturer's manual using ELISA reading device ICN Flow Titerk Multiscan Plus Mk11 (Labsystems, Espoo, Finland).

Then, in all serum samples Hp concentration was evaluated. Hp was determined using guaiacol (peroxidase) test according to Jones and Mould [17]. The method is based on the measuring peroxidase activity of a Hp-methemoglobin complex. Amount of decomposed peroxide is proportional to the concentration of Hp. Colorless guaiacol is oxidized by decomposing peroxide to the yellow tetraguaiacol and the absorbance of the final solution is red by the wave length of 492 nm.

Median of Hp concentration was calculated for sera with detectable levels of Hp separately for CAE-positive and CAE-negative goats. Moreover, Mann-Whitney U test was applied to evaluate statistical significance of a difference between Hp concentrations in CAE-positive and CAE-negative sera. All statistical analyses were performed in PASW Statistics 18.0.0 (IBM SPSS).

## Results

Out of 325 serum samples 146 (45%) were positive and 179 (55%) were negative in CAE-ELISA. Totally, 11 serum samples were positive for Hp – 4 CAE-positive (2.7%) and 7 CAE-negative (3.9%). Concentration of Hp in Hp-positive samples is given in Table 1. Medians of Hp concentrations in CAE-positive and CAE-negative sera with detectable level of Hp were 1.06 g/l and 0.49 g/l, respectively. Results of Mann-Whitney U test confirmed that there was no statistically significant difference between Hp concentrations in CAE-positive and CAE-negative sera.

## Discussion

The method of Hp determination based on guaiacol peroxidase was initially invented on sheep but it can be used in goats [7].

The panel of goats sera used in our study was not only very numerous but also has been selected in a proper way to ensure high credibility of obtained results. Nevertheless Hp could be detected only in 11 serum samples (out of 325 samples used in a study) what indicates that Hp is rather absent in normal goat serum. It remains in concordance with information given by Petersen *et al.* [5] for cattle.

Hp is considered one of the main APP in ruminants [5], although some studies suggest its minor role in wild ruminants [10]. Its basic concentration is very low, approximately 0.02 g/l in cattle [4] but Petersen *et al.* [5] claim that it is not present in normal bovine serum. Hp rises in the presence of acute inflammation in any part of the body to reach even 2 g/l within a few days. Such reaction was detected in cattle with concentrations above 0.4 g/l in reticuloperitonitis [11] and in small ruminants. Sheep experimentally infected with *Corynebacterium pseudotuberculosis* showed increase of serum Hp concentration up to 1.65 g/l within a week [8]. In experimental pregnancy toxemia Hp reached the highest point after 3 days [7]. There are no data about APP role in SRLV infection in goats.

Hp concentration of 0.1 g/l is usually assumed as a cut-off. All values below 0.1 g/l are physiological and values above cut-off suggest inflammatory condition (similarly to cattle the concentrations 0.1-0.4 g/l, 0.4-1.0 g/l and above 1.0 g/l may be assumed as mild, moderate and severe inflammation, respectively [18, 19, 20].

No statistically significant difference between Hp concentrations in two studied groups could be identified. The result accords with the observation made for SRLV-infect-

**Table 1.** Hp concentration in Hp-positive serum samples

Hp-positive serum number	CAE-ELISA result	Hp concentration [g/l]
1	positive	0.17
2	positive	0.83
3	positive	1.28
4	positive	9.25
5	negative	0.11
6	negative	0.16
7	negative	0.19
8	negative	0.49
9	negative	0.62
10	negative	0.91
11	negative	1.21

ed lambs [21]. Although high concentration of Hp was detected by Ulutaş *et al.* [9] in nematode and liver fluke infections in goats, which are rather chronic conditions, there are some reports which indicate that Hp is more typical for acute than chronic inflammations. In experimental bacterial infections Hp concentration tended to fall gradually to reach the baseline within a few weeks post infection [8, 22], as it did also in the chronic phase of mastitis in dairy cows [12]. In cattle Hp was elevated in many viral and bacterial infections such as calf bronchopneumonia, infectious bovine rhinotracheitis, bovine viral diarrhea, metritis but most of them were acute indeed [5]. Serum amyloid A seems to remain elevated much longer than Hp and it seems to be a better indicator of chronic inflammation [23]. On the other hand, elevated concentrations of various APP are detected in chronic arthritis [24]. Moreover, median of Hp concentration in goats with detectable levels of Hp in CAE-positive group was more than 2-fold higher than in CAE-negative one. CAE is a chronic progressive disease which can be perceived as the series of consecutive damaging stimuli [5] and in such diseases APP tend to wane and wax [23]. Of course as very small number of goats showed elevation in Hp it might be caused by incidental inflammation, totally unrelated to CAE. However, in light of the fact that SRLV infection is associated with permanent stimulation of lymphocytes [25], the difference in medians should raise doubts about the incidental background of the rise in Hp concentration. Given that the examined seropositive goats were infected for different periods of time and this variable could not be analyzed in this study, it seems reasonable to evaluate the dynamics of Hp concentrations in subsequent stages of SRLV infection in the future.

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