Serum Total and Lipid-Bound Sialic Acid Concentrations in Sheep with Natural Babesiosis

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Abstract

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The aim of this study was to determine the serum concentrations of total and lipid-bound sialic acid in sheep naturally infected with *Babesia ovis* before and after treatment. Thirty diseased sheep and ten control animals were used. Babesia infection was confirmed with Giemsa's staining of blood smears. Compared to the control animals (1.351 ± 0.100 mmol·l^{-1}; 0.385 ± 0.001 mmol·l^{-1}), a marked increase of serum total and lipid bound sialic acid concentrations (1.929 ± 0.101 mmol·l^{-1}; 0.479 ± 0.004 mmol·l^{-1}, respectively) was obtained in infected sheep ($p < 0.05$). One week after treatment, serum total and lipid bound sialic acid concentrations significantly decreased (1.554 ± 0.005 mmol·l^{-1}; 0.411 ± 0.02 mmol·l^{-1}, respectively) ($p < 0.05$). Despite this decline, total and lipid bound sialic acid concentrations of treated animals were still significantly increased in sera compared to the control animals ($p < 0.05$). When the infected animals were treated with an antiparasitic drug (diminazene aceturate), the serum total and lipid bound sialic acid levels had a tendency to decrease to the normal ranges. It can be concluded that *Babesia ovis* infection induced marked and persistent elevations of serum total and lipid-bound sialic acid concentrations, suggesting that these indicators would indirectly promote the invasion and presence of the parasite in the host.

Sheep, Babesia ovis, sialic acids

Sialic acids are nine-carbon monosaccharides that link to the terminal galactose, N- acetylgalactosamine, or to other sialic acids in carbohydrate chains of glycoproteins or glycolipids (Corfield and Schauer 1982). Sialic acids usually occupy exposed terminal positions on the oligosaccharide chains of glycoconjugates and frequently serve as ligands for receptors such as selectins and siglecs, which mediate a variety of cell-cell adhesion processes in the inflammation and in the immune response (Kelm and Schauer 1997; Malykhy et al. 2001).

Babesia species are protozoan parasites that develop in the erythrocytes of vertebrate hosts and are transmitted transovarially and transstadially by ixodid ticks (Urquhart et al. 1996; Friedhoff 1988). The severity of the disease caused by these parasites, namely babesiosis, is related to the vertebrate host and protozoan species (Urquhart et al. 1996). Ovine babesiosis is the most important haemoparasitic tick-borne disease of small ruminants caused by *Babesia ovis*, *Babesia motasi* and *Babesia crassa*. These parasites are widespread in tropical and subtropical areas of the world (Uilenberg 2001). *Babesia ovis* is highly pathogenic especially in sheep, and causes severe infections that are charecterised by fever, anaemia, icterus and haemoglobinuria (Almeria et al. 2001).

Sialic acids are present in normal human and animal serum. It has been demonstrated that sialic acid concentrations are elevated in patients suffering from various diseases (Schutter et al. 1992; Wongkham et al. 2003). However, the effect of *Babesia ovis* infection on serum total and lipid-bound sialic acid content in sheep has not been reported previously.

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Therefore, the aim of the present study was to examine if there is any relationship between babesiosis and the serum total and lipid-bound sialic acid concentrations.

Materials and Methods

Forty Akkaraman sheep with a body mass of 25 - 30, at the age of 4 - 5 years and localized in different regions of Van between June and August of 2005 were used as subjects for this study. Thirty sheep were infected naturally with *Babesia ovis* and ten control animals were clinically healthy. All the cattle included in this study submitted to clinical and parasitological examinations. Treatment of thirty infected animals was conducted with diminazene aceturate (Berenil®) 7% solution) once at a dose of 3.5 mg/kg**,** intramuscularly. Blood samples of all animals were taken into plain and EDTA containing vacutainer tubes from the jugular vein. Samples were firstly taken at the onset of the disease during the disease season of babesiosis (June - August), then one week following the treatment of the diseased animal. At this time, the treated animals did not show any clinical signs of babesiosis. Serum samples were obtained after centrifugation at 1700*g* for 15 minutes at room temperature, aliquoted and stored at -20 °C until used.

Serum total sialic acid concentration was determined by a method previously described by Sydow (1988). Lipid bound sialic acid concentration was determined by the method described by Katopodis et al. (1982). The amount of total and lipid bound sialic acid were determined by use of a standard curve developed from a standard sample of n-acetyl neuraminic acid.

EDTA-blood was used to prepare thin blood smears. Blood smears were fixed in methanol, stained with Giemsa and examined for the presence of blood protozoa.

The results were expressed as means ± standard deviation. Duncan's test was used for statistical analysis, setting $p < 0.05$ to establish statistically significant differences.

Results

Table 1. Serum total sialic acid and lipid-bound sialic acid concentrations in healthy sheep (control group) and naturally infected sheep with *Babesia ovis* (infected group) at the onset of the disease and one week after treatment by diminazene aceturate parasites (mean ± S.D.)

Indicators	Control group (n = 10)	Infected group (n = 30)	
		During disease	After Treatment
Total sialic acid ($mmol \cdot l^{-1}$)	1.351 ± 0.100	1.929 ± 0.101*	1.554 ± 0.005*
Lipid-bound sialic acid ($mmol \cdot l^{-1}$)	0.385 ± 0.001	0.479 ± 0.004*	0.411 ± 0.02*

* ($P < 0.05$)

The biochemical findings obtained in the study are summarised in Table 1.

Blood smears prepared from the thirty diseased animals showed the presence of piroplasm of *Babesia ovis* in the red blood cells with different parasitaemia. On the other hand, no piroplasm was detected in control animals. After recovery (i.e. following the treatment), piroplasms were still detected in all infected animals by direct microscopic examination with very low degree of parasitaemia.

Mean serum total and lipid bound sialic acid concentrations of the diseased animals were significantly higher than in the control and treated animals ($p < 0.05$). A significant decline was observed in the serum total and lipid bound sialic acid concentrations of the treated animals ($p < 0.05$). Total sialic acid and lipid bound sialic acid concentrations of treated animals (one week after treatment) were still significantly increased in sera compared to the control animals ($p < 0.05$).

Discussion

This study was conducted to examine the effect of babesia infection on total and lipid-bound serum sialic acid concentrations. Babesia infection induced a significant increase of total and lipid-bound serum sialic acid concentrations. Furthermore, these acid

concentrations were significantly reduced after the treatment of the animals. Nevertheless, despite the significant decline in serum total and lipid bound sialic acid content following the treatment, their concentrations remained significantly elevated up to one week following the treatment. It is well known that after recovery, animals become carriers of the parasite (Kaufmann 1996) and such high concentrations of sialic acid were probably due to the carrier state of treated animals.

It is currently unknown how infection with *Babesia ovi*s leads to the increase of serum sialic acid content. However, infections with various parasites such as leishmania (Chatterjee et al. 1998; Karagenç et al. 2005), and trypanosoma (Eslevo et al. 1982; Olaniyi et al. 2001) are also associated with elevated serum sialic acid concentrations. It was reported that the serum total and lipid-bound sialic acid concentrations were found to be significantly higher in cattle infected with blood parasites (theileria, anaplasma) as compared to the control group (Ertekin et al. 2000).

Host sialic acids were reported to play an important role in the erythrocyte invasion by babesia parasites (Yokoyama et al. 2006). Total sialic acid plays a role as a host receptor in the erythrocyte invasion by *Babesia bovis,* and treatment prevented the increase of sialic acid (Gaffar et al. 2003). In addition, Okamura et al. (2005) observed that sialic acid residues on host RBC play important roles in the erythrocyte infections by *Babesia caballi* and *Babesia equi* and cause a significant increase in its concentration. Conversely, sialic acid deficiency in the erythrocytes of infected cattle with *Babesia bovis* has been reported by Commins et al. (1988).

Sialic acid could modulate biological cell-cell interactions in two non-mutually exclusive ways. First, sialic acid could mask the underlying sugar chains (i.e. lactosaminic sequences), hindering then from interacting with galactose-specific lectins (galectins) (Razi and Varki 1998). Second, sialic acid would directly interact with specific sialic acid-binding lectins (siglecs) (Dall'olio 2000). Therefore, increased contents of sialic acid would interfere with the attachment of sporozoites on host cells, or promote the invasion of erythrocytes by merozoites.

Our finding showed that natural infection of sheep with *Babesia ovis* leads to significant increases of serum total and lipid-bound sialic acid concentrations, suggesting that sialic acids would indirectly promote the invasion and persistence of parasite in the host.

Koncentrace celkové a na tuk vázané kyseliny sialové v séru ovcí přirozeně infikovaných babesiózou

Cílem této studie bylo určit koncentrace celkové a na tuk vázané kyseliny sialové v séru ovcí přirozeně infikovaných *Babesia ovis* před a po léčbě. Použito bylo třicet nemocných a deset kontrolních ovcí. Infekce babesiemi byla prokázána Giemsovým barvením krevních nátěrů. Ve srovnání s kontrolními zvířaty (1.351 ± 0.100 mmol·l^{-1}; 0.385 ± 0.001 mmol·l^{-1}) byly u infikovaných zvířat zaznamenány výrazně vyšší koncentrace celkové a na tuku vázané kyseliny sialové (1.929 ± 0.101 mmol·l^{-1}; 0.479 ± 0.004 mmol·l^{-1}) ($p < 0,05$). Jeden týden po léčbě tyto koncentrace výrazně klesly (1.554 ± 0.005 mmol·l^{-1}; 0.411 ± 0.02 mmol·l^{-1}) ($p < 0,05$). Ačkoliv koncentrace celkové a na tuk vázané kyseliny sialové v séru léčených zvířat klesly, byly ve srovnání s kontrolními zvířaty stále signifikantně zvýšené ($p < 0,05$). Když byla infikovaná zvířata léčena antiparazitiky, měly koncentrace celkové a na tuk vázané kyseliny sialové v séru tendenci klesnout k fyziologickým hodnotám. Z výsledků pokusu vyplývá, že infekce *Babesia ovis* indukovala výrazné a stálé zvýšení koncentrace celkové a na tuk vázané kyseliny sialové v séru, což naznačuje, že tyto ukazatele by mohly nepřímo odhalovat infekci a přítomnost parazita v hostiteli.

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