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Elevated serum total sialic acid concentrations in sheep with peste des petits ruminants

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Summary

Peste des petits ruminant (PPR) is a highly contagious and economically important disease of small ruminants. Plasma sialic acid is a marker of the acute phase response in pathological conditions and significant alterations within total sialic acid serum (TSA) concentrations have been documented in various diseases. The purpose of the study was to investigate changes in TSA serum concentrations in sheep suffering from peste des petits ruminants. Eighty sheep sera were tested for antibodies against PPRV with c-ELISA and 22 of the 80 sera (27.5%) were found to be positive. Twenty two sheep naturally infected by PPRV aged between 1-2 years old were categorized as the infected group and 16 clinically healthy sheep of the same age constituted the control group. Venous blood was sampled from the sheep's jugular veins. The concentration of TSA serum was measured by Warren's thiobarbituric acid assay. Clinical, hematological and biochemical changes, including liver function tests, were also evaluated. Higher concentrations of TSA serum were found in the infected sheep (84.7±13.1 mg/dL) compared to the healthy sheep (63.5±4.7 mg/dL), ($P \leq 0.001$). In addition, significant correlations were determined between TSA and clinical symptoms, hematological changes and liver function tests of the infected sheep. The findings of the study indicate that TSA plays a part in the disease processes of PPR and that determining TSA serum concentrations may be used as a supplementary laboratory test in conjunction with clinical and laboratory findings when evaluating the prognosis of PPR.

Keywords: sialic acid, peste des petits ruminants, sheep

Peste des petits ruminants (PPR) is a highly contagious and infectious disease of domestic and wild small ruminants caused by PPR virus (PPRV) (3). PPR is characterized by fever, gastroenteritis, pneumonia, conjunctivitis, necrotic stomatitis and purulent ocular and nasal discharges (14). It is economically important disease of sheep and goats and has high morbidity and mortality with a wide distribution in the Middle-East, Asia and sub-Saharan Africa (6, 7).

Sialic acid (SA), also known N-acetylneurominic acid (NANA) is carbohydrate component of cell membran glycolipids and have a central roles in cell-to-cell recognition and interaction, transport and receptor functions of membranes and stability, and survival of blood glycoproteins in biological systems (13). Serum sialic acid has been proposed as a marker of the induced acute-phase response (18) since many acute-phase proteins are sialylated at the terminus of the oligosaccharide chain of the glycoprotein (12). Elevated serum total sialic acid (TSA) concentrations have been documented in several diseases such as enzootic bovine hematuria (15), bovine leukosis (17), bovine leptospirosis (4), rheumatoid artrhitis (20), diabetes (22), renal disease (9) and cancer (5). But there is no report available on the serum TSA in sheep with PPR. Therefore, the present study has been undertaken to investigate the effect of PPR on serum TSA concentration in sheep.

Material and methods

Animals. Twenty two sheep naturally infected by PPRV aged between 1-2 years old were categorized infected group and 16 clinically healthy sheep were included as a control group as same age. All infected animals had clinical signs of the disease, including fever, erosive stomatitis, gastroenteritis and conjunctivitis. The clinical diagnosis confirmed by serologically (see below). The samples were taken in the study before sheep had seen PPR symptoms almost 2 weeks ago. Sheep were not have PPR infection even before and were not vaccinated against PPR.

Serological analyses. Competitive enzyme linked immunosorbet assay (cELISA) were used to detect antibodies against PPRV as described in the manual of pestes des petits ruminants enzyme linked immunosorbent assay. All procedures were carried out according to the instructions in the manual included with Office International des Epizooties Manual of Standards (1).

Hematological and biochemical analyses. Blood was collected from jugular vein into EDTA-containing tubes for hematological and empty tubes for biochemical analysis. Blood cell counts were counted by cell-counter (Ermax-18, Erma Inc., Japan). Serum was removed by centrifugation at 1550 g for 10 min. of tubes with clothing blood. Sera were separeted and

Tab. 1. Clinical findings, blood cell counts and blood chemistry in infected and healthy sheep (X ± Sx)

Parameters	Infected group (n = 22)	Control group (n = 16)
Body temperature (°C)	40.07 ± 0.8***	38.5 ± 0.3
Respiratory rate (breaths/min.)	40.2 ± 4.9***	27.2 ± 4.7
Heart rate (beats/min.)	115.5 ± 7.8***	95.2 ± 6.5
Leukocyte (× 10^3/µL)	5.1 ± 0.4**	5.6 ± 0.5
Lymphocyte (× 10^3/µL)	2.7 ± 0.3**	3.2 ± 0.5
Monocyte (× 10^3/µL)	1.4 ± 0.1**	1.2 ± 0.3
Neutrophil (× 10^3/µL)	0.9 ± 0.04	0.8 ± 0.1
Eosinophil (× 10^3/µL)	0.3 ± 0.03	0.4 ± 0.03
Total protein (g/dL)	7.2 ± 0.3*	6.8 ± 0.5
Albumin (g/dL)	2.3 ± 0.2**	2.7 ± 0.4
Globulin (g/dL)	4.9 ± 0.4**	4.2 ± 0.8
Albumin/globulin ratio	0.48 ± 0.07**	0.68 ± 0.24
Total bilirubin (mg/dL)	0.33 ± 0.12*	0.22 ± 0.05
Direct bilirubin (mg/dL)	0.23 ± 0.08*	0.16 ± 0.04
Indirect bilirubin (mg/dL)	0.10 ± 0.05*	0.05 ± 0.02
Cholesterol (mg/dL)	108.1 ± 11.3	106.6 ± 14.3
AST (U/L)	40.7 ± 10.2**	29.9 ± 4.2
ALT (U/L)	11.2 ± 4.0*	8.2 ± 3.0
GGT (U/L)	19.7 ± 2.6**	17.3 ± 3.0

Explanations: * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$ vs. control

Tab. 2. Correlation coefficients between TSA and clinical, hematological and biochemical characteristics in infected and control sheep

Parameters	Infected (n = 22)	Control (n = 16)
	Corr. coeff.	Corr. coeff.
Body temperature (°C)	.959**	–.386
Respiratory rate (breaths/min.)	.745**	–.282
Heart rate (beats/min.)	.633**	–.326
Leukocyte (× 10^3/µL)	–.551**	–.345
Lymphocyte (× 10^3/µL)	–.732**	–.546*
Monocyte (× 10^3/µL)	.660**	.362
Neutrophil (× 10^3/µL)	–.189	.368
Eosinophil (× 10^3/µL)	–.085	–.403
Total protein (g/dL)	.551**	–.096
Albumin (g/dL)	–.775**	–.235
Globulin (g/dL)	.773**	.044
Total bilirubin (mg/dL)	.734**	.412
Direct bilirubin (mg/dL)	.708**	.391
Indirect bilirubin (mg/dL)	.693**	.333
Cholesterol (mg/dL)	–.291	.095
AST (U/L)	.791**	.035
ALT (U/L)	.864**	.310
GGT (U/L)	.664**	.313

Explanations: * $p \leq 0.05$, ** $P \leq 0.01$

samples were stored at –70°C until assays. Serum concentrations of total protein, albumin, total bilirubin, direct bilirubin, cholesterol, and activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl-transferase (GGT) were analysed using commercially available kits (Sigma-Aldrich Chemie GmbH, Eschenstraße 5,82024 Taufkirchen, Germany) according to the maufacturer's instruction by autoanalyser (Autolab, AMS Srl, Selective Access). Globulin and indirect bilirubin concentrations were obtained by subtracting the concentrations of albumin and direct bilirubin from total protein and total bilirubin concentrations, respectively. Serum TSA concentration was measured by colorimetric Warren's method (21).

Statistical analyses. Mann-Whitney *U*-test for two independent samples was used to determine whether significant differences exist between the infected and control group for clinical finding, blood cell count and blood chemistry (11). Correlation analysis between the parameters was performed by Pearson's correlation test. Data are expressed as mean ± standard deviation. Results were considered as significant when p values were less than 0.05.

Results and discussion

Serological findings. Eighty sheep sera were tested to antibodies against PPRV with c-ELISA and 22 of 80 sera (27.5%) were found positive.

Hematological and biochemical findings. The tab. 1 presents the clinical, hematological and biochemical findings of infected and healthy sheep. Clinical findings were significantly higher in infected group than in control group ($p \leq 0.001$). Blood leukocyte and lymphocyte numbers of infected sheep showed significantly declinations in comparison to healthy sheep ($p \leq 0.01$). On the contrary, the blood monocyte numbers of infected animals showed significant increases ($p \leq 0.001$). However, no significant alterations in the number of blood neutrophils and eosinophils was observed.

Compared to healthy sheep, significant increases the mean serum concentrations of total protein ($p \leq 0.05$), globulin ($p \leq 0.01$), total bilirubin, direct bilirubin, indirect bilirubin ($p \leq 0.05$) and activities of AST ($p \leq 0.01$), ALT ($p \leq 0.05$) and GGT ($p \leq 0.01$) of the infected sheep were determined. Infected sheep had a significantly lower albumin concentration compared to healthy sheep ($p \leq 0.01$). The serum albumin/globulin ratio in the infected sheep was significantly lower ($p \leq 0.01$) than that of the healthy (tab. 1). No significant differences were present between the infected and control sheep for serum cholesterol concentration. Serum TSA concentration in infected group was significantly higher than that in the control group ($p \leq 0.001$) (fig. 1).

The results in tab. 2 showed that TSA were positively-correlated with body temperature, respiratory rate, heart rate, monocyte, total protein, globulin, total bilirubin, direct bilirubin, indirect bilirubin, AST, ALT and GGT. TSA were negatively-correlated with leukocyte, lymphocyte and albumin. There was no significant correlation between the TSA and neutrophil, eosinophil and cholesterol.

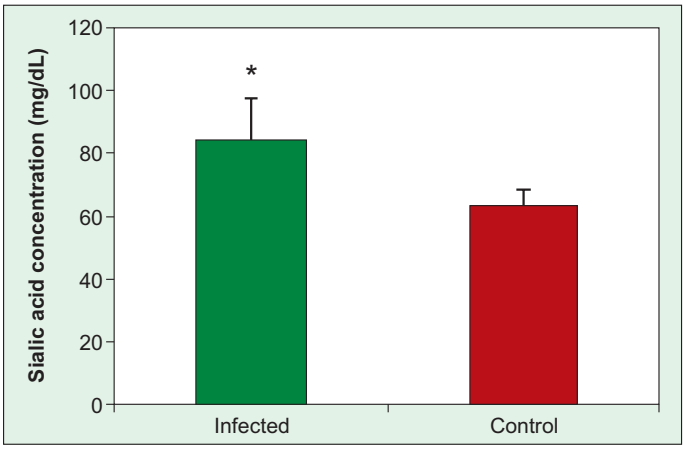

Fig. 1. Serum sialic acid concentrations (X ± Sx) in infected and control sheep vs. control
Explanation: * $p \leq 0.001$

Peste-des-petits-ruminants is important diseases of small ruminants causing great economic losses due to their high morbidity and mortality and threats national livestock industries (6). PPR infection is still major problem as sporadic outbreaks among sheep, goats and wild small ruminants (1). The disease is characterized by digestive and respiratory system disorders such as erosive stomatitis, gastroenteritis and pneumonia (2). In present study, the infected sheep also showed the same clinical signs. Toplu (19) reported that multifocal areas of coagulative necrosis and vacuolation of hepatocytes in sheep naturally infected with PPRV. Ours data showed that the infected sheep also had abnormal liver function tests. This finding is agreement with Toplu's data (19) and indicate that the liver function tests are useful in the prognosis of PPR. The total and differential leukocyte counts serve as indicators for pathogenesis of lymphotropic viruses like PPRV. In the present study, moderate leukopenia, lymphopenia and monocytosis were mainly hematological abnormalities in infected animals. The results are partially in agreement with a similar report on virus induced immune suppression in PPRV (10). The monocytosis would be related to immune response to PPR infection. However, not significant alterations were observed for eosinophil and basophil numbers in infected animals.

Sialic acids are important component of cell membrane glycoproteins and glycolipids (8). Serum sialic acid concentration has been found to be elevated in chronic liver diseases, malignant tumors and bacterial infections (4, 5, 16). Sialic acid concentration in serum has been considered as a marker of acute phase reactants which contain sialic acid residues of its oligosaccharide side chain (18). Data obtained this study show that serum TSA concentration in infected sheep was significantly higher ($p \leq 0.001$) from the healthy sheep. The elevation of TSA concentration may be explained by increased acute phase reactions associated with PPR infection. Results of the present study also revealed an inverse relationship between TSA and clinical, hematological and biochemical findings which including liver function tests. The correlation of serum TSA and liver function tests suggesting a possible role of hepatic damage in the elevation of serum TSA in PPR infection of sheep. When combined with other markers, TSA concentrations are helpful in disease screening and follow-up, as well as in monitoring of prognosis. It is thought that an increase in concentration of TSA is most likely related to induced cell-mediated immune response, acute phase reaction and hepatic damage that accompanies PPR.

In conclusion, serum TSA concentration increases correlated with clinical and laboratory signs in PPR infection in the sheep. The results suggest that serum TSA concentration can be used in the prognosis of PPR as an supplementary laboratory test in combination with clinical and laboratory findings.

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