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Short Communication

Serum Sialic Acid Levels in Calves with Pneumonia

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Abbreviations: LBSA, lipid-bound sialic acid; PBSA, protein-bound sialic acid; SA, sialic acid; TSA, total sialic acid

INTRODUCTION

Pneumonia is a common and frustrating disease of farm animals caused by many infectious agents, either viruses or bacteria, or in many cases both. One of the previous study in this region reported that *Mannheimia haemolytica* and *P. multocida* were responsible for 56.6% of pneumonia in calves (Gokce *et al.*, 1997). Detection and definition of pneumonia is based on clinical examination. Laboratory examinations of blood chemistry during the course of pneumonia are needed for better definition, treatment and prognosis. One such component was thought to be total serum sialic acid concentration. Sialic acid is a group name for acetylated derivates of neuraminic acid. *N*-Acetylneuraminic acid is a common terminal sugar unit of the oligosaccaride of glycoproteins and glycolipids, is cell-surface constituents commonly found in bacteria and animals (Schauer, 1982).

Serum Sialic acid (SA) concentrations have been studied by many researchers in diseases such as nephrotic syndrome, rheumatoid arthritis (Schauer, 1982; Stefenelli *et al.*, 1985), pneumonia (Gottschalk, 1972), theileriosis, anaplasmosis (Ertekin *et al.*, 2000) and bovine leptospirosis (Keles *et al.*, 2000) and concentrations of SA are reported to be increased in these diseases. It has been reported that, at the beginning of inflammatory reactions or in injury, serum SA concentrations increase rapidly (Haq *et al.*, 1993). However, the underlying mechanism that causes increase in serum SA has not been clearly defined. Serum SA may be a marker of the acute-phase response, since serum concentrations were significantly related to established acute-phase proteins such as alpha-1 acid glycoprotein (Taniuchi *et al.*, 1981; Stefenelli *et al.*, 1985; Haq *et al.*, 1993). Acute-phase reactants influence total sialic acid concentrations because of their glycoprotein structure (Taniuchi *et al.*, 1981). It has been suggested that serum SA concentrations, especially of lipid-bound sialic acid, are an indicator of inflammatory diseases (Motoi, 1984).

Although, pneumonia events have been extensively documented, there is no study reporting the changes in serum SA levels in pneumonia in ruminants. The aim of this study was to determine the clinical biochemical importance of sialic acid levels (total, lipid-bound and protein-bound) in calves with pneumonia and total protein, albumin and globulin levels for the diagnosis of the disease.

MATERIAL AND METHODS

Animals

The study included 10 calves with pneumonia and 10 healthy Montofon calves aged between 3 and 12 months. Pneumonic calves that were admitted to the Clinics of Veterinary Medicine and control animals were from the farm of the Veterinary Faculty the University of Kafkas.

Clinical examination and blood sampling

Accurate diagnosis of pneumonia in each calf was made by clinical examination. Venous blood (10 ml) from the jugular vein was collected into a plain tube for analysis, and blood samples were centrifuged at 3000*g* for 10 min at room temperature. Serum were separated and stored at -25°C until analysis.

Serum sialic acid analysis

Serum total sialic acid (TSA) and lipid-bound sialic acid (LBSA) analyses were carried out colorimetrically using a spectrophotometer (UV-1201, Shimadzu, Japan) according to the methods reported by Sydow (1985) and Katopodis and Stock (1980). The quantity of protein bound sialic acid (PBSA) was calculated by subtracting the quantity of LBSA from that of TSA.

Biochemical analysis of serum samples

Serum total protein and albumin values were measured using an autoanalyser (Ektachem DT 60 II, DTCS II, Kodak, USA). The globulin concentration was calculated by subtraction of the albumin value from the total protein value.

Statistical analysis

The analysis of data was carried out using the SPSS (1993) statistical program. The significance level was set at $p < 0.05$. Normal distribution of the data was tested using the Anderson–Darling normality test. Values were expressed as mean $\pm$ SD. To determine test specificity and sensitivity, the cut-off value was set as mean TSA and LBSA values

TABLE I
The concentrations (means ± SE) of sialic acid and biochemical parameters in healthy calves ($n = 10$) and calves with pneumonia ($n = 10$)

Parameter	Calves with pneumonia	Healthy calves	Significance
Total sialic acide (mg/dl)	$99.24 \pm 3.48^{***}$	70.68 ± 4.33	$p < 0.001$
Lipid-bound sialic acide (mg/dl)	$59.45 \pm 4.0^{***}$	36.74 ± 1.44	$p < 0.001$
Protein-bound sialic acide (mg/dl)	39.78 ± 4.49	33.93 ± 3.80	NS
Total protein (g/L)	$81.9 \pm 1.9^{***}$	62.20 ± 0.80	$p < 0.001$
Albumin (g/L)	$31.9 \pm 0.64^{**}$	28.00 ± 0.68	$p < 0.01$
Globulin (g/L)	$50.00 \pm 1.27^{***}$	34.60 ± 0.70	$p < 0.001$
Albumin/globulin ratio	$0.63 \pm 0.01^{***}$	0.84 ± 0.04	$p < 0.001$

$^{**}p < 0.01$, $^{***}p < 0.001$; NS, not significant

plus the relevant SD (70.68 + 13.70 = 84.38 for TSA; 36.74 + 4.57 = 41.3 for LBSA). Yates–corrected chi-squared test was used to compare the proportion of TSA- and LBSA-positive calves in both groups.

RESULTS

The concentrations of serum SA, total protein, albumin and globulin and albumin/globulin ratios are given in Table I. Serum TSA concentrations were 70.68 ± 4.33 mg/dl in the healthy calves and 99.24 ± 3.48 mg/dl in pneumonic calves. LBSA levels of healthy and diseased calves were 36.74 ± 1.44 mg/dl and 59.45 ± 4.00 mg/dl, respectively. TSA and LBSA levels were significantly higher in diseased calves than in healthy calves ($p < 0.001$). PBSA concentrations in healthy calves and pneumonic calves were 33.93 ± 3.80 mg/dl and 39.78 ± 4.49 mg/dl, respectively.

Mean total protein levels in pneumonic calves (81.90 ± 1.9 g/L) were significantly higher than in healthy calves (62.20 ± 0.80 g/L) ($p < 0.001$). Similarly, serum albumin concentrations in calves with pneumonia (31.90 ± 0.64 g/L) were higher than those in healthy calves (28.00 ± 0.68 g/L). Serum globulin concentrations and albumin/globulin ratio in healthy calves were 34.60 ± 0.70 g/L and 0.84 ± 0.04, and in pneumonic calves were 50.00 ± 1.27 g/L, 0.63 ± 0.01 ($p < 0.001$), respectively.

Normality testing revealed that values of TSA ($p = 0.3$), LBSA ($p = 0.09$) and PBSA ($p = 0.363$) in healthy calves and of TSA ($p = 0.94$), LBSA ($p = 0.153$) and PBSA ($p = 0.862$) in calves with pneumonia were normally distributed. When the cut-off point was set at 84.38 for TSA, the sensitivity was 100% and specificity was 80%. The proportion of healthy and pneumonic cattle positive for TSA differed significantly ($\chi^2 = 10.2$, $p < 0.001$). As for LBSA, when the cut-off value was set at 41.3, the sensitivity and specificity were 100% and 90%, respectively. The proportion of LBSA-positive healthy and pneumonic calves also differed significantly ($\chi^2 = 12.9$, $p < 0.001$).

DISCUSSION

In this study the levels of SA were determined in calves with pneumonia and in healthy animals. Studies have reported that serum SA levels alter with, age, sex and pregnancy (Dezan *et al.*, 2002; Wakabayashi and Masuda, 2004; Maghzal *et al.*, 2005). Serum TSA and LBSA levels in pneumonic calves were significantly higher than in healthy calves ($p < 0.001$). Serum biochemical analysis of SA shows a positive correlation between pneumonia and the presence of inflammation. It is likely that part of the SA is covalently bound to the acute-phase glycoproteins, which results in increased serum SA after the development of pneumonia. The results are in agreement previous results of Motoi (1984), Ertekin and colleagues (2000) and Keles and colleagues (2000).

It is well documented that inflammation and injury result is a rapid increase is SA levels (Stefenelli *et al.*, 1985; Haq *et al.*, 1993). Therefore, determination of serum SA levels, especially LBSA levels, was an important indicator of inflammation due to infectious and non-infectious agents (Motoi, 1984). This finding explains the increased SA levels in pneumonic calves. Because during pneumonia immune mechanisms are activated and synthesis of globulin and sialoproteins by liver is stimulated (Schauer, 1982; Thougaard *et al.*, 1998).

Additionally, the glycoprotein structure of increased acute-phase reactants might have influenced serum TSA levels (Taniuchi *et al.*, 1981). Thus, increased SA may be an indicator of increased acute-phase reactants (Taniuchi *et al.*, 1981; Stefenelli *et al.*, 1985; Haq *et al.*, 1993). Another important cause of increased SA has been reported to be bacteria and viruses which play an important role in the development of pneumonia. These agents are known to increase the activity of extracellular neuraminidase and break the bond between SA and the cell membrane (McCuller and Bartmess, 2003). This study attempted to elucidate the potential role of sialic acid as a blood chemistry parameter in bovine pneumonia. The results suggest that serum SA might be a useful parameter as an indicator of inflammation in pneumonia.

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