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Short Communication / *Kısa Bilimsel Çalışma*

Decreased serum sialic acid, albumin-globulin ratio and total protein levels in cattle heavily infected with *Theileria annulata*[*]

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Summary: The present study was designed to determine the changes in the levels of serum total sialic acid and total protein, albumin/globulin ratio in naturally infected cattle with tropical theileriosis for a better understanding of the pathogenesis of tropical theileriosis. Eight cattle heavily infected (50-70% parasitamia) with *Theileria annulata* had significantly lower serum sialic acid, total protein, albumin and globulin levels and albumin-globulin ratios than uninfected eight control cattle. Levels of serum sialic acids were found to be 133.87±0.77 mg/dl and 194.12±3.91 mg/dl in cattle with theileriosis and healthy animals respectively. These values between two groups found to be significant ($p<0.001$). Levels of serum total protein were found to be 5.70 ± 0.17 g/dl and 7.00 ± 0.14 g/dl in cattle with theileriosis and healthy animals respectively. These values between two groups found to be statistically significant ($p<0.001$). Levels of serum albumin were found to be 2.16 ± 0.04 g/dl and 3.02 ± 0.04 g/dl in cattle with theileriosis and healthy animals respectively. These values between two groups found to be statistically significant ($p<0.00$). Levels of serum globulin were found to be 3.53 ± 0.15 g/dl and 3.97 ± 0.12 g/dl in cattle with theileriosis and healthy animals respectively. These values between two groups found to be statistically significant ($p<0.05$). Albumin-globulin ratio in serum were found to be 0.61 ± 0.02 and 0.76 ± 0.02 in cattle with theileriosis and healthy animals respectively. These values between two groups found to be statistically significant ($p<0.002$). These findings suggest that levels of serum proteins are usually decreased significantly in Tropical theileriosis, which may be the result of liver damage. In paralel to decreased serum proteins, sialisation of proteins in tropical theileriosis may be the result of decreased glycoproteins during the disease process. These results may be useful to evaluate the prognosis and treatment of the disease with the other laboratory findings in clinic.

Key words: Sialic acid, total protein, albumin:globulin ratio, cattle, *Theileria annulata*

Theileria annulata ile yüksek oranda enfekte sığırlarda azalan serum sialik asit, albumin-globulin oranı ve toplam protein düzeyleri

Özet: Çalışmada, sığırlarda tropikal theileriosis'in patogenezis yönünden daha iyi anlaşılabilmesi için serum sialik asit, toplam protein, albumin ve globulin düzeyleri ile albumin-globulin oranları araştırılmıştır. Enfekte olmayan (kontrol) sekiz sığır ile karşılaştırıldığında, *Theileria annulata* ile yüksek oranda enfekte (%50-70 parazitemi) olan sekiz hasta sığırda serum sialik asit, toplam protein, albumin ve globulin düzeyleri ile albumin-globulin oranlarının önemli derecede düştüğü belirlenmiştir. Serum sialik asit ortalama değerleri sağlıklı sığırlarda 194.12 ± 3.91 mg/dl, hasta sığırlarda ise 133.87 ± 0.77 mg/dl olarak tespit edilmiş, gruplar arası farklılık önemli bulunmuştur ($p<0.001$). Serum toplam protein ortalama değerleri sağlıklı olanlarda 7.00 ± 0.14 g/dl, hastalarda ise 5.70 ± 0.17 g/dl olarak tespit edilmiş, gruplar arası farklılık önemli bulunmuştur ($p<0.001$). Serum albumin ortalama değeri ise sağlıklı ve hastalıklı olanlarda sırasıyla 3.02 ± 0.04 ve 2.16 ± 0.04 g/dl hesaplanmış ve farkın istatistik önemli olduğu saptanmıştır ($p<0.000$). Serum globulin ortalamaları sırasıyla sağlıklı olanlarda 3.97 ± 0.12 g/dl, hastalarda 3.53 ± 0.15 g /dl olarak tespit edilmiş, gruplar arası farklılığın istatistik önemli olduğu hesaplanmıştır ($p<0.05$). Serum albumin-globulin oranı ortalaması sağlıklı grupta 0.76 ± 0.02, hastalıklı grupta ise 0.61 ± 0.02 olarak bulunmuş ve farkın istatistik önemli olduğu tesbit edilmiştir ($p<0.002$). Bu sonuçlar, serum proteinlerinin önemli derecede azaldığını göstermektedir. Serum proteinlerindeki bu azalış karaciğer hasarının bir sonucu olabilir. Önemli derecede azalan protein düzeylerine paralel olarak, tropikal theileriosis'de proteinlere bağlı düşük sialik asit düzeyleri de hastalığın gelişimi süresince glikoproteinlerdeki azalışın bir sonucu olabilir. Bu sonuçların hastalığın prognoz ve tedavisinin izlenmesinde klinikteki diğer laboratuvar bulguları ile birlikte değerlendirilmesinin faydalı olabileceği kanaatine varılmıştır.

Anahtar sözcükler: sialic acid, toplam protein, albumin:globulin oranı, sığır, *Theileria annulata*.

[*] Theileriosis'li sığırlarda serum albumin-globulin oranı ve sialik asit düzeyleri başlıklı yüksek lisans tezinden özetlenmiştir.

Tropical theileriosis is a cattle disease caused by the protozoan *Theileria annulata*. The disease has been seen in North Africa, the Mediterranean basin, through the Middle East to the Indian sub-continent and China (6, 20). Tropical theileriosis has been reported earlier in different regions of Turkey (8, 10, 22). *T. annulata* is propagated in cattle with stage-to-stage transmission by *Hyalomma ticks* (2, 19). The main pathological damage in cattle is induced by the schizont stage (19).

Tropical theileriosis is among the most serious constraints on the livestock industry in the regions where it occurs. It threatens exotic *Bos taurus* breeds of European origin in particular and it may cause 40-60% mortality (3). Infection by Theileria parasites limits the movement of cattle between countries and can result in production losses and high mortality in susceptible animals. Therefore, it has greatest economic impact.

Plasma proteins are mainly synthesised by the liver. Analysis of total protein concentrations and percentage of protein fractions are important in various disease states (12). Glycoproteins are defined as proteins which contain glycan chains linked glycosidically to selected amino acid residues. Monosaccharides commonly found in the glycans of the glycoproteins including N-acetylneuraminic acid, sialic acid (11). Sialic acids as monosaccharides are link to the terminal galactose, N-acetylgalactosamine, or to other sialic acids in carbohydrate chains attached to glycoproteins and glycolipids (5). Sialic acids are often involved in important cell surface communications and infection processes. Particularly, sialic acids are present in normal serum of human and animals that their content in serum has been changed in various diseases (1, 4, 9, 14, 16).

The main objective of this study was to examine the levels of serum total sialic acid and total protein, albumin:globulin ratio in cattle heavily infected with *Theileria annulata* and compare it with the results of uninfected animals for a better understanding of the pathogenesis of tropical theileriosis.

Samples of the study were obtained from eight *Theileria annulata* naturally infected and eight healthy Holstein heifer belong to Ankara region in Turkey. All examined one year old cattle included in this study went through a physical and parasitological examination on microscope.

All of the diseased animals showed some or all of the clinical signs of theileriosis including fever, enlargement of lymph nodes, inappetence, drooling from mounth, serous nasal discharge and swelling of the eyelids. However, eight animals (Holstein heifer) showed high percentage of parasitamia (50-70%), determined by microscopic examination of blood cells following Giemsa dye method, were particularly selected to analyse the levels of serum total sialic acid and total protein, albumin and globulin levels in one year old Holstein cattle. Piroplasm parasitaemia was recorded as the number of piroplasm-infected erythrocytes in 100 cells. Serum total protein and albumin levels were determined by Biuret (17) and Brom Cresol Green (BCG) (7) methods respectively.

Serum total sialic acid levels were measured by Sydow's method (24). For this purpose, 400 µl of serum were mixed with 3 ml of 5% perchloric acid for 5 min at 100 ^{o}C and centrifuged at 1400 g for 4 min, 2 ml of supernatant were also mixed with 400 µl of Echrlich reagent (p-dimethylaminobenzaldehyde). Following incubation at 100 ^{o}C for 15min, 2ml of distilled water were added on samples and the optical density of samples at 525nm were read using a spectrophotometer (Shimatzu). A standard curve was obtained using known quantities of freshly prepared N-acetylneuraminic acid (Sigma) solution in water..

Statistical analysis; Differences between uninfected and infected animal groups for serum sialic acid, total protein, albumin, globulin and albumin:globulin ratio were analysed by the Mann-Whitney U test.

Table 1. Comparison and statistically significance (P) of serum sialic acid, total protein, albumin and globulin levels and albumin-globulin ratios between uninfected (control) and infected cattle with *Theileria annulata.*

Tablo 1. *Theileria annulata* ile enfekte ve enfekte olmayan (kontrol) sığırlarda serum sialik asit, toplam protein, albumin ve globulin oranlarının istatistik önemi (P) ve karşılaştırılması.

Analysed parameters	*Uninfected cattle*			*Infected cattle*			P
	n	x	Sx	n	x	Sx	
Sialic acid (mg/dl)	8	194.12	3.91	8	133.87	0.77	0.001***
Total protein (g/dl)	8	7.00	0.14	8	5.70	0.17	0.001***
Albumin A (g/dl)	8	3.02	0.04	8	2.16	0.04	0.001***
Globulin G (g/dl)	8	3.97	0.12	8	3.53	0.15	0.050*
A/G ratio	8	0.76	0.02	8	0.61	0.02	0.002**

*p<0.05 **p<0.01 ***p<0.001

Serum of eight healthy and eight *Theileria annulata* infected cattle at a parasitaemia with 50% to 70% were used individually to analyse sialic acid, total protein, albumin and globulin levels and albumin:globulin ratio. Table 1 shows the results of the study. Cattle naturally infected with *T. annulata* had significantly lower ($p<0.001$) serum sialic acid in comparison to normal control cattle. Serum total protein and albumin levels of cattle infected with *T. annulata* were decreased significantly

($p<0.001$) compared to uninfected control cattle. Globulin levels of serum from cattle infected with *T. annulata* were decreased significantly ($p<0.05$) in comparison to uninfected control cattle. Albumin: globulin ratio were also decreased significantly ($p<0.002$) in cattle with theileriosis than control cattle.

Most of the previous studies on biochemical parameters in *Theileria annulata* infection have been carried out on experimentally infected calves (21, 23, 25). There is only one study published on biochemical profiles in adult (>1year) and young (<1year) Friesian cattle naturally infected with *T. annulata* in Saudi Arabia (18). The work described here was designed to determine the changes in the levels of serum total sialic acid and total protein, albumin:globulin ratio in eight naturally infected one year old cattle (Eight Holstein heifer with high percentage (50-70%) of parasitamia) with tropical theileriosis belong to Ankara region in Turkey. Karagenç and her colleagues (13) were observed the increased levels of serum sialic acid in acute theileriosis with low percentage of parasitemia. Interestingly, opposite to this result, we have observed a significant decrease in serum sialic acid levels of naturally infected one year old cattle with high parasitemia (50-70%) of *T. Annulata*. These two conradictory results may be the result of acute and chronic theileriosis with different percentage of parasitemia rates seen in young and older cattle serum samples which were analysed separately in two diferent study. Here, we also report the significant decreases in serum total protein, albumin and globulin levels and albumin-globulin ratio as well as significantly decreased serum sialic acid levels of naturally infected one year old cattle with tropical theileriosis than uninfected cattle (see Table 1). Except for the albumin-globulin ratio, some of the findings of the present study, decreased concentrations of serum total protein, albumin and globulin of cattle naturally infected with *T. annulata,* were found to be consistent with the results of Omer and colleagues (18). The major site of synthesis of the plasma proteins is the liver (12). Severe tissue damage in the liver occurs during the *T. annulata* infections of cattle (15). It could be explained that the decreased serum total protein concentration in cattle naturally infected with *T. annulata* was possible due to hypoalbuminaemia and hypoglobulinaemia arising from liver failure.

In conclusion, values of the all examined parameters were decreased significantly in one year old cattle with chronic tropical theileriosis in comparison to uninfected cattle. These findings suggest that levels of serum proteins is usually decreased in tropical theileriosis, which may be the result of liver damage seen in theileriosis. In paralel to decreased serum proteins, sialisation of proteins may be the result of decreased glycoproteins during the disease process. These results may be useful to evaluate the prognosis and treatment of the disease with the other laboratory findings in clinic as well as to better understanding of the pathogenesis of tropical theileriosis.

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