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Evaluation of Serum Cobalt, Copper, Iron, Calcium, Phosphorus and Magnesium Concentrations in Cattle Naturally Infected with *Theileria annulata*

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Absract: The purpose of this study was to evaluate serum albumin, glucose, calcium (Ca), cobalt (Co), copper (Cu), iron (Fe), magnesium (Mg), and phosphorus (P) in cows with naturally infected with *Theileria annulata*. The material of this study was a total of 15 cattle with different ages, breeds and genders diagnosed as theileriosis according to clinical and microscopical examination. According to statistical analyses of biochemical parameters; serum glucose, Ca, Co, Cu, Fe, Mg, and P concentrations were detected significantly low in cattle with theileriosis (P<0.001). In conclusion; mineral substance levels were altered in cattle naturally infected with *Theileria annulata*. Additionally to the classical treatment of theileriosis, administering mineral supplements including Ca, Co, Fe, Cu, Mg, and P to the animals is thought to be more useful in treatment.

Keywords: Cattle, Mineral substance, *Theileria annulata*.

Theileria annulata ile Doğal Enfekte Sığırlarda Serum Kobalt, Bakır, Demir, Kalsiyum, Fosfor ve Magnezyum Konsantrasyonlarının Değerlendirilmesi

Öz: Bu çalışmanın amacı, doğal *Theileria annulata* ile enfekte sığırlarda serum albümin, glikoz, Kalsiyum (Ca), kobalt (Co), bakır (Cu), demir (Fe), Magnezyum (Mg) ve Fosfor düzeylerini değerlendirmektir. Bu çalışmanın hayvanın materyali olarak klinik ve mikroskobik inceleme sonuçlarına göre theileriozis tanısı konulan değişik yaş, ırk ve cinsiyette 15 sığır kullanıldı. Biyokimyasal parametrelerin istatistiki analizine göre serum glikoz, Ca, Co, Cu, Fe, Mg ve P konsantrasyonları theileriozisli sığırlarda önemli düzeyde düşük tespit edildi (P<0.001). Sonuç olarak; Doğal Theileria annulata ile enfekte sığırların mineral madde düzeyleri değişmektedir. Theileriozisin klasik tedavisine ek olarak Ca, Co, Fe, Cu, Mg ve P içeren mineral preparatların verilmesinin tedavide daha fasydalı olacağı düşünülmektedir.

Anahtar Kelimeler: Mineral madde, Sığır, *Theileria annulata*.

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INTRODUCTION

Bovine tropical theileriosis caused by *Theileria annulata*, is a tick-borne disease of cattle. Theileria disease is important disease that is widespread in tropical and subtropical climates countries caused morbidity and loss of productivity in indigenous cattle and lethal disease in imported high-grade cattle and crossbred stock (1-4).

Macro and micronutrients are inorganic substances essential to maintain the normal function and living status in domestic animals. These nutrients play a critical role in physiological processes related to health, growth and reproduction, and the adequate function of the immune and endocrine systems (5). Cobalt is the main component of vitamin B_{12} which plays an important role in energy metabolism, haematological parameters and amino acid metabolism (6-8). Cobalt has been used for years to prevent unproductiveness with signs of anorexia, low body weight gain, emaciation, low milk yield and anaemia (7). It is reported that theres is closely between Concentration copper (Cu) and health in the cows (9). Copper is a compound of enzyme catalysts and participates in important biochemical function (6). Copper is responsible for activation of numerous vital enzyme and its deficiency causes various disorders (8). Iron (Fe) is essential to virtually s as a component of hemoglobin and numerous other iron-containing proteins in the body. Fe deficiency caused the incidence of infectious diseases and impairment of the activities of iron-containing enzymes in cells of the immune system in animals (10). Magnesium (Mg) is an important mineral for in animals. Mg should be sufficient to metabolic activity and protect animal health (11). In cattle with theileriosis, there were decreased serum calcium and phosphorus concentrations in cattle infected with *T. annulata* in the present study which was also decreased in the previous studies (12). Col and Uslu (13) reported that hypocalcaemia in their study could be due to hypoproteinemia, decreased dietary intake, intestinal malfunction and kidney damage. The decreased phosphorus serum concentration could be due to diarrhea and renal wasting (14). Recent reviews have focused on the role of trace minerals in disease resistance in ruminants (5-7). The purpose of this study is to present changes in serum mineral concentrations in cattle with theileriosis before and after treatment, and importance of these concentration changes in treatment and prognosis of the disease.

MATERIALS and METHODS

Animal selection and blood collection

The material of this study was a total of 15 cattle with different ages, breeds and genders diagnosed as theileriosis according to clinical and microscopical examination and 10 healthy cattle as control group. All animals in the study adhered to the principle of local Ethics Committee (Date and decision of the ethics report: 01.08.2013 and 2013/08). Clinical findings of the animals were evaluated systemically before treatment and at the 7th day after treatment. In order to perform microscopical examination, blood samples were obtained from V. auricularis before and after treatment and blood smears were prepared and stained with Giemsa. Patients with piroplasm form of theileria in their erytrocytes were evaluated as positive.

Blood samples were obtained from V. jugularis before and at the 7th day of treatment and for once from the control group for haemotological and biochemical analyses. In order to perform hematological analyses, blood samples were obtained to tubes containing EDTA and to perform biochemical analyses, serum was extracted from blood samples obtained to anticoagulant free tubes which were centrifuged on 3000 RPM (Rotofix 32 – Hettich). Obtained serum samples were preserved in -20 ˚C until evaluation. Single dose of buparvaquon (Butalex – Ceva DİF) were administered to infected

animals with dose of 2.5 mg/kg live weight. Oxytetracyclin (Geosol injectable solution/VETAŞ Turkey) was administered for 5 days intramuscularly in order to prevent secondary infections.

Hematological and Biochemical Examination

Hematocrit (Hct), hemoglobin (Hb) concentration and White Blood Cell amount (WBC) were measured with hemogram device (QBC vetautoreader® – Idexx). Serum Cobalt (Co), Copper (Cu), Phosphorus (P), Iron (Fe), Calcium (Ca) and Magnesium concentrations were measured with Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Thermo Scientific, Designed in UK and Made in China). Serum glucose concentrations were measured spectrophotometrically (Photometer 5010 – Boehringer Mannheim) according to the test procedures.

Statistical Analysis

Statistical analysis was performed using SPSS 20 for Windows. Clinical signs, hematological and biochemical parameters were stated as Mean, Standart Deviation, Minimum and Maximum values. Kruskal-Wallis test was performed for comparing these attributes. Spearmen-correlation coefficient was calculated in order to determine the relation between parameters in each group. Statistically significance level was taken as 5 %.

RESULTS

Clinical Findings

The clinical examinations of cattle with theileriosis; anorexia, dullness, swelling in superficial lymph nodes, hyperemia in visible mucosa and conjunctivas were observed. Anemia and petechial hemorrhage were observed in certain animals (Table1).

Table1. Clinical findings in healthy cattle and cattle infected with *Theileria annulata*.
Tablo 1. Sağlıklı ve *Theileria annulata* ile enfekte sığırlarda klinik bulgular.

Parameters	Control n=10 $\overline{X} \pm$ SD	Before treatment n=15 $\overline{X} \pm$ SD	7th day After Treatment n=15 $\overline{X} \pm$ SD
Body Temperature (ºC)	38.32±0.12[a]	40.26±0.26[d]	38.36±0.10[a]
Heart Rate (beat /min)	73.50±1.20[a]	84.30±4.48[c]	80.32±3.05[a]
Respiratory Rate (breath / min)	27.50±0.56[a]	43.30±1.84[b]	35.48±1.38[d]

Importance of statistical difference in the same lines were determined as ab: P<0.001, ac: P<0.01 ve ad: P<0.05.

Hematological Findings

Hemoglobin concentration, hematocrite and total leucocyte amount were significantly lower in cattle suffering from theileriosis than in the healthy controls (P<0.001). Despite treatment, while hematological parameters of cattle with theileriosis were detected close to same hematological parameters of healthy cattle; Htc and WBC values were detected statistically lower than healthy cattle (P<0.01 and P<0.05, respectively) (Table 2).

Table 2 Hematological findings in healthy cattle and cattle with Theileriosis.
Tablo 2. Sağlıklı ve Theileriozisli sığırlarda hematolojik bulgular.

Parameters	Control n=10 $\overline{X} \pm$ SD	Before treatment n=15 $\overline{X} \pm$ SD	7th day After Treatment n=15 $\overline{X} \pm$ SD
Hemoglobin concentration (g/dl)	11.14±0.24[a]	7.12±0.54[b]	9.86±0.24[a]
Hematocrit (%)	33.10±0.58[a]	16.22±0.40[b]	26.84±0.32[c]
Total Leucocyte Count (x10^9/L)	8.88±0.42[a]	5.10±0.44[b]	7.22±0.82[d]

Importance of statistical difference in the same lines were determined as **ab**: P<0.001, **ac**: P<0.01 ve **ad**: P<0.05.

Biochemical Findings

There is a decrease in serum albumin concentrations in the cattle infected with *T. annulata*. There was, however, no significant difference in the albumin ratio between infected and healthy cattle (P>0.05). Serum glucose, P, Ca, Co, Fe, Cu and Mg concentrations in cattle with theileriosis were detected significantly lower than control group before treatment (P<0.001). Despite the treatment; serum glucose (P<0.01), Co (P<0.01), Cu (P<0.01), Fe (P<0.05), Mg (P<0.01) and P (P<0.01) concentrations were detected lower in cattle with theileriosis at the 7th day of treatment than the same parameters of control group (Table 3).

Table 3 Biochemical parameters in healthy cattle and cattle with Theileriosis.
Tablo 3. Sağlıklı ve Theileriozisli sığırlarda biyokimyasal parametreler.

Parameters	Control n=10 $\overline{X} \pm$ SD	Before treatment n=15 $\overline{X} \pm$ SD	7th day after treatment n=15 $\overline{X} \pm$ SD
Glucose (mg/dl)	72.32±6.70[a]	46.24±7.27[b]	62.41±4.34[c]
Albumin (gr/dl)	2.82±0.25[a]	2.66±0.32[a]	2.70±0.34[a]
Ca (mg/dl)	8.21±2.30[a]	4.50±1.63[b]	7.78±0.68[a]
Co (µg/L)	51.36±4.10[a]	20.44±2.10[b]	46.76±3.66[c]
Cu (µg/L)	1130.50±246.43[a]	528.62±61.16[b]	858.75±115.56[c]
Fe (mg/L)	1.70±0.35[a]	0.41±0.17[b]	1.14±0.22[d]
Mg (mg/dl)	2.57±0.25[a]	1.26±0.34[b]	1.87±0.16[c]
P (mg/dl)	6.30±0.68[a]	2.605±0.52[b]	4.51±0.56[c]

Importance of statistical difference in the same lines were determined as **ab**: P < 0.001, **ac**: P < 0.01 ve **ad**: P < 0.05.

DISCUSSION and CONCLUSSION

Significant changes in cattle infected with *Theileria annulata* were reported in several studies (3, 13-16). In the present study; RBC count, PCV and Hb concentration in cattle with theileriosis were significantly lower than healthy ones (P<0.05). With progression of parasitemia severity, a significant decrease in RBC count, PCV and Hb concentration was observed (P<0.05). These findings were similar to those of many researchers (3,15,16). The decline in RBC count, PCV and Hb concentration can also be attributed to multiplication of piroplasms in RBC which results in erythrolysis (2).

In the present study, there was a decrease in serum glucose concentrations in cattle infected with *T. annulata* compared with control group. The findings were in accordance with the findings of researchers (13,14) as they reported the decreased serum glucose concentration could be due to glucose utilization in blood and hepatic dysfunction as a result of theileria infection. Kincaid and colleagues (17) reported the low serum total protein

concentration in cattle naturally infected with *T. annulata* was possible beacuase of hypoalbuminemia and hypoglobulinemia resulting from liver failure and severe lymphocytopenia. In current study serum albumin concentration in infected with *T. annulata* were found to be numerically lower concentrations of healthy cattle.

The decreased serum copper and iron concentrations in Theileria-infected cattle could be attributed to the inability of the damaged liver to synthesize ceruloplasmin and transferrin, respectively (12). In this study, serum Fe and Cu concentrations were detected significantly lower than control group (P<0.001). Copper has roles on superoxyde dismutase and cytochrome oxidase, myelin synthesis and keratinisation (18). Cytochrome C oxidase enzyme which has important function in energy conversion is dependent on copper (19). Therefore copper is an essential element and primarily has role in carbohydrate metabolism (6). Researchers (19) reported plasma Cu concentrations as 90-150 ug/dl for ruminants. In this study; serum Cu levels in healthy cattle were determined as 1130.50±246.43 µg/L. Before treatment, serum Cu levels in cattle with theileriosis were determined as 528.62±61.16 µg/L. At the 7th day after treatment, serum Cu level in theileriosis was 858.75±115.56 µg/L. Copper has roles on growth, regulating the reproduction and carbohydrate metabolism. Decrease in food intake resulted by anorexia lead to impairment of carbohydrate metabolism (6,9,18,19) is also thought to cause decrease in Cu levels in cattle with theileriosis.

In this study; before treatment, while average serum calcium concentrations were detected lower in cattle with theileriosis than control group; serum albumin level did not change. Decreased calcium concentrations in theileriosis could be resulted from decreased dietary intake, intestinal malfunction, kidney damage and decreased phosphorus concentration due to diarrhea and renal wasting (12,13).

Mg has great impact on basic metabolic rate in fat and carbohydrate metabolism, and blood glucose regulation (20). Serum magnesium concentration of healthy cattle is reported as 1.2-3.5 mg/dl (21). In the current study; when Mg levels of cattle with theileriosis before treatment were compared with control group; Mg levels in bovine with theileriosis was found lower (P<0.001). In this study; the reasons of lower magnesium concentration are thought as increase in tubular resorption and loss of appetite due to gastrointestinal atony.

Serum phosphorus level in healthy cattle is reported as 5.6-6.5 mg/dl. As reported, low food intake by these factors lead to phosphorus deficiency (22). Hypophosphataemia causes various clinical findings such as anorexia, pica, muscle weakness, intravascular haemolysis impaired liver function (23).

In this current study, the serum phosphorus concentrations were significantly lower in cattle suffering from theileriosis than in the healthy controls (P<0.01). it has been postulated that the lower level of phosphorus amount in cows with theleriosis could partly be due to the anorexia condition prevailing in the disease. These datas support the researchers' datas (22,23). In the study, the decrease in the concentration of Mg and P in cattle with theleriosis can be caused diarrhoea and wasting (12).

In conclusion; serum Ca, Co, Cu, Fe, Mg and P concentrations in cattle with *Theileria annulata* were detected low before treatment. Additionally to the classical treatment of theileriosis, administering mineral supplements to the animals is thought to be more useful in treatment. Therefore there should be more studies performed about the contribution of minerals to the treatment of theileriosis. This study will provide an insight to the studies will be performed in this respect.

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