

Evaluation of Cobalt, Vitamin B₁₂ and Homocystein levels in Cattle infected with *Theileria annulata*

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Abstract

This study was conducted to assess the pattern of changes and the relative values of homocysteine in bovine tropical theileriosis. The diseased group comprised of 20 cattle (2–3 years old) naturally infected with *Theileria annulata*. As control group, 10 uninfected cattle were also sampled. While cobalt (Co), vitamin B₁₂, folate, total protein (TP), globulin values were determined significantly lower than control group (P<0.001), serum homocystein (Hcy), creatine kinase-myocardial band (CK-MB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH), creatinine, urea, and blood urea nitrogen (BUN) concentrations were determined higher than control group (P<0.001). As a result, decreases in serum B₁₂ vitamin concentrations of cattle with theileriosis lead to increases in serum Hcy level.

Keywords: Cattle; *Theileria annulata*; Homocystein; Cobalt; Vitamin B₁₂

Introduction

Bovine tropical theileriosis caused by *Theileria annulata*, is a tick-borne disease of cattle. The disease is widespread across Asia, North Africa, and Europe, causing morbidity and loss of productivity in indigenous cattle and lethal disease in imported high-grade cattle and crossbred stock (Khalil *et al.* 2011; Singh *et al.* 2001; Nazifi *et al.* 2009).

Symptoms are enlargement of local lymph glands, fever, haemorrhages on visible mucous membranes and sometimes on skin, anaemia and jaundice particularly (Oryan *et al.* 2013) in subacute and chronic forms of the disease (Omer *et al.* 2003). Oedema and enlargement of, and haemorrhages in lymph glands and spleen, haemorrhages in subcutis and on most of the serous and mucous membranes on endocardium, pericardium and epicardium, and ulcers in abomasum which rarely extend to intestines, oesophagus, tongue and gums are common necropsy findings in bovine theileriosis (Nazifi *et al.* 2009).

Cobalt is required for rumen microorganisms to synthesis vitamin B₁₂. Besides homocystein requires vitamin B₁₂ for methylation and re-synthesizing methionine. Therefore cobalt/vitamin B₁₂ deficiency is considered to cause decrease in plasma methionine concentration and increase in homocysteine concentration (Stangl *et al.* 1998). Vitamin B₁₂ is essential for normal growth and erythrocyte formation. Vitamin B₁₂ is synthesized in rumen by bacterias when cobalt is present (Issi *et al.* 2010).

Homocystein is an intermediary amino acid which does not exist in proteins and formed by methionine metabolism (Rezaei and Dalir-Naghadeh, 2009; Gauthier *et al.* 2003; Lin *et al.* 2003). Homocystein is a sulfhydryl which contains amino acid produced by methionine demethylation. (Hajjar, 2001; Chai and Abramsm, 2001). Methionine is an essential amino acid produced by breakdown of endogenous proteins and additionally by breakdown of diet proteins (Gauthier *et al.* 2003). Homocystein leads to pathological disorders due to lowering vitalness of nitric oxide, increasing thrombocyte and collagen amount for vascular endothelium and give rise to excessive production of homocystein reactive molecules (homocystein-tiolacton) (O'Grady *et al.* 2002). Homocystein parameter is valuable for the diagnosis and follow up of cobalamine or folate deficiencies, and it has been accepted as an independent risk factor for premature cardiovascular disease.

The purpose of this study is to investigate whether Hcy levels have changed in cattle infected with *Theileria annulata*, which is characterized by blood and circulatory disorders.

Material and Method

The material of this study consisted of sick animals brought to animal hospital of Yuzuncu Yil University Veterinary Faculty and 30 farm animals (20 with theileriosis and 10 healthy) from the villages of Ozalp, Van.

In order to diagnose the disease in addition to clinical findings, blood was obtained from auricular vein and blood films were prepared and stained with Giemsa. Diagnosis was performed according to microscopical examination of blood films by observing piroplasm form of the agent in infected erythrocytes. In blood films, 100 red blood cells (RBC) were counted and percentage of RBCs infected with piroplasm form of *T.annulata* was evaluated and parasitemia level was determined. Morphological changes of the agents were evaluated in blood films before treatment and at the 7th day after treatment. Single dose of buparvaquone 2.5mg/kg body weight intramuscularly was administered on the cervical region of the cattle diagnosed as theileriosis. Oxytetracyclin (Geosol injectable solution/VETAS Turkey) was administered for 5 days intramuscularly in order to prevent secondary infections. At the 7th day after treatment, in order to evaluate hematological and biochemical parameters, blood was obtained from jugular vein. Anticoagulant tubes were used in order to evaluate hematological parameters, and to evaluate biochemical parameters anticoagulant free tubes were used

Hematocrite, hemoglobin concentration and leucocyte count were evaluated by using QBC device (QBCvet autoreader®-Idexx). Obtained blood samples were centrifuged in 3000 RPM/10 minutes and serum and plasma were extracted. Biochemical parameters were evaluated. Serum total protein (TP), albumin, globulin, glucose, blood urea nitrogen (BUN), creatinine, aspartate amino transferase (AST), alkaline phosphotase (ALP), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine kinase (CK) and creatine kinase-myocardial band (CK-MB) values were determined by auto analyser device (Cobas-6000 c501- Roche-Hitachi). Serum vitamin B₁₂ and folate levels were examined by means of Cobas-6000 e601 (Roche-Hitachi Japan) hormone analyzer. Homocysteine levels, on the other hand, were determined by means of an ELISA equipment (ELISA reader®-DAS) and through the use of a commercial homocysteine test kit (Human Homocysteine ELISA Kit-CUSABIO, Catalog Number; CSB-E13814h), at 450 nm wavelength as advised in the user manual of the test kit.

Serum Cobalt concentration was evaluated in healthy cattle and cattle with theileriosis by using ICP-M device (Thermo Scientific, Designed in UK and Made in China) in Directorate of Scientific Application Center in University of Yuzuncu Yil.

Statistical Analysis

Descriptive statistics, Mean, Standart Deviation were defined as Minimum and Maximum values. Kruskal-Wallis test was used for comparing groups for these properties. Spearman coefficient of correlation was calculated for determining relationship between these properties individually in the groups. Statistical significance level was taken as 5% and for calculations SPSS software was used.

Results

Clinical Findings

According to clinical examination before treatment; anorexia, dullness, enlargement of superficial lymph nodes, hyperemia on visible mucosa and conjunctiva. In some patients, anemia and petechial hemorrhages were observed on mucosa and conjunctivas (Table 1).

Table 1: Clinical Findings in Cattle with Theileriosis

	Before Treatment (n=20) ($\bar{X} \pm SD$)	7 th day After Treatment (n=20) ($\bar{X} \pm SD$)	Control (n=10) ($\bar{X} \pm SD$)
Body temperature (°C)	40.26±0.26 ^d	38.36±0.10 ^a	38.32±0.12 ^a
Heart rate (beat/min)	84.30±4.48 ^c	80.32±3.05 ^a	73.50±1.20 ^a
Respiratory rate (breaths/ min)	43.30±1.84 ^b	35.48±1.38 ^d	27.50±0.56 ^a

Hematological Findings

Hematological parameters of healthy cattle with infected cattle's same parameters; statistically hemoglobin, hematocrite and total leucocyte count were determined significantly lower ($P < 0.001$). At the 7th day after treatment, hematological parameters of cattle infected with theileriosis were closed to the same hematological parameters of healthy cattle. However Htc and WBC values were statistically lower than healthy cattle's at the same parameters ($P < 0.01$ and $P < 0.05$, respectively)(Table 2).

Table 2: Hematological Findings of Healthy Cattle and Cattle with Theileriosis

Parameters	Before treatment (n=20) ($\bar{X} \pm SD$)	7 th day After Treatment (n=20) ($\bar{X} \pm SD$)	Control (n=10) ($\bar{X} \pm SD$)
Hemoglobin (g/dl)	6.70±0.58 ^b	8.14±0.18	10.16±0.28 ^a
Hematocrite (%)	14.2±0.40 ^b	22.94±0.34 ^c	32.10±0.68 ^a
Total Leucocyte Count ($\times 10^9/L$)	4.12±0.46 ^b	6.32±1.08 ^d	8.78±0.32 ^a

Biochemical findings

When evaluating statistical analyses of biochemical parameters; while Co, Vitamin B₁₂, Folate, TP, Globulin concentrations were significantly lower than control group before treatment ($P < 0.001$), serum HCy, CK-MB, ALP, ALT, AST, CK, LDH, Creatinin, Urea and BUN concentrations were found higher ($P < 0.001$). However before and at the 7th day after treatment, serum albumin concentrations were not significant when compared to control group ($P > 0.05$). While Co ($P < 0.01$), Vitamin B₁₂ ($P < 0.01$), Folate ($P < 0.01$), Globulin ($P < 0.01$) concentrations were lower at the 7th day after treatment, serum HCy ($P < 0.01$), CK-MB ($P < 0.01$), ALP ($P < 0.01$), ALT ($P < 0.01$), AST ($P < 0.01$), CK ($P < 0.01$), GGT ($P < 0.01$), LDH ($P < 0.01$), Creatinine ($P < 0.01$), Urea ($P < 0.01$), BUN ($P < 0.01$) concentrations were significantly higher than control group. Serum TP and Albumin concentrations of cattle with theileriosis at the 7th day after treatment were determined closer to the control values.

Table 3: Biochemical Parameters of Cattle with Theileriosis and Healthy Cattle

Parameters	Before Treatment (n=20) ($\bar{X} \pm SD$)	7 th day After Treatment (n=20) ($\bar{X} \pm SD$)	Control (n=10) ($\bar{X} \pm SD$)
HCy (pg/ml)	19.33±9.68 ^b	8.19±0.58 ^c	5.70±1.81 ^a
Co (mg/L)	.05±.01 ^b	.08±.01 ^c	.11±.01 ^a
Vitamin B ₁₂ (pg/ml)	169.13±36.54 ^b	368.42±120.38 ^c	868.59±410.63 ^a
Folate (ng/ml)	5.38±1.20 ^b	10.30±2.08 ^c	16.36±2.52 ^a
CK-MB (U/L)	153.67±58.22 ^b	58.99±8.69 ^c	34.68±10.45 ^a
ALP (U/L)	69.05±31.48 ^b	33.55±5.19 ^c	18.40 ±7.04 ^a
ALT (U/L)	21.20±7.27 ^b	6.73±1.23 ^c	4.20±0.69 ^a
AST (U/L)	188.15±82.25 ^b	62.53±15.41 ^c	28.10±6.86 ^a
CK (U/L)	205.85±133.35 ^b	133.70±22.89 ^c	123.033±94.95 ^a
LDH (U/L)	1259.85±199.19 ^b	847.90±108.25 ^c	460.00±115.57 ^a
Creatinine (mg/dL)	1.17±0.25 ^b	0.85±0.06 ^c	0.65±0.08 ^a
Urea (mg/dL)	40.16±9.31 ^b	25.17±3.11 ^c	15.10±2.18 ^a
BUN(mg/dL)	18.75±4.31 ^b	11.80±1.40 ^c	7.10±1.20 ^a
TP(g/dl)	5.92±.71 ^c	7.17±0.34	7.74±0.82 ^a
Albumin (g/dl)	2.76±.36	2.80±0.38	2.92±0.29
Globulin(g/dl)	3.15±.75 ^b	4.20±0.41 ^c	5.1±0.35 ^a

Discussion

Although there are effective treatments and common vaccination applications against theileriosis in our country, the disease still maintains its importance. The disease leads to death and important economic losses especially in highly productive breed. Clinical findings in this study are parallel with several researchers' reports (Basbug and Gul, 2011; Gokce *et al.* 1998; Pasa, 2008).

In the present study, the RBC count, PCV, and Hb concentration in cattle with theileriosis were significantly lower than healthy cattle ($P < 0.05$). Also, on the onset of parasitemia severity, a significant decrease in RBC count, PCV, and Hb concentration was detected ($P < 0.05$). These findings were similar to researchers (Nazifi *et al.* 2009; Khalil *et al.* 2011; Ramin *et al.* 2011). The decline in RBC, PCV, and Hb can also be attributed to multiplication of piroplasm in RBC which results in RBC lysis (Singh *et al.* 2001). Besides, the decrease in Hb and PCV was considered to occur due to anorexia resulted by Co deficiency leading to inadequate synthesis of Vitamin B₁₂ and Folate (Stangl *et al.* 2000).

While AST activities were significantly higher in cattle before treatment than control group; decrease was found at the 7th day after treatment. Increase in AST activities in the cattle with theileriosis was considered to occur due to the severity of liver damage. These data are supported by several researchers' reports (Gokce *et al.* 1998; Issi *et al.* 2010; Omer *et al.* 2003).

At the 7th day after treatment Average creatinine concentration of cattle with Theileriosis were significantly higher than control group. Increase in creatinine concentration before treatment might be in the result of sensitivity stated by researchers in their reports (Pasa, 2008; Basbug and Gul, 2011).

Several researches reported that serum albumin concentrations in cattle with Theileriosis were in normal reference ranges (Riondet *et al.* 2008; Pasa, 2008). In this study, before and at the 7th day after treatment determining serum albumin concentration in cattle in normal reference values confirm the data of the study (Pasa, 2008).

Before treatment, increase in serum LDH enzyme concentration was determined. Increase in LDH activity in the cattle with Theileriosis is considered to occur due to LDH release from erythrocytes resulted by hemolysis (Basbug and Gul, 2011). Besides, in the study serum ALP and ALT activities were higher than control group. Increase in these enzyme activities are considered due to the liver damage stated by researchers (Issi *et al.* 2010).

Serum TP and globulin values of cattle with theileriosis were significantly lower at the 7th day after treatment than control group ($P < 0.001$). The decrease in these parameters were considered to occur anorexia resulted by severity and time of the disease and excessive albumin break down due to increase in metabolic needs in febrile diseases. Besides BUN and urea concentration of cattle with theileriosis were determined higher than healthy cattle. This increase in BUN and urea was resulted due to decrease in food intake (Pasa, 2008) and kidney damage progressing during disease (Kızıllı *et al.* 2007).

Our study shows that cattle suffering from theileriosis had significantly lower concentrations of cobalt and vitamin B₁₂ in their serum as compared to control groups and additionally, a lower cobalt concentration was present in infected cattle with lower PCV. The present study showed that the cattle suffering from theileriosis had significantly lower concentrations of cobalt and vitamin B₁₂ in their sera. Reduced cobalt and vitamin B₁₂ levels cause increased Hcy which is in accordance with those reported by others (Kozat, 2011).

Animals with cobalt deficiency both showed decrease in hemoglobin concentration and hematocrite. There is a positive relationship between Hb and Htc changes with plasma cobalt levels. Decrease in plasma cobalt concentration leads to decrease in Hb, Htc, folate and vitamin B₁₂ concentrations and leads to increase in homocystein levels (Stangl *et al.* 2000). Ruminants require cobalt in order to synthesis vitamin B₁₂ and when there is adequate amount of cobalt, vitamin B₁₂ is able to be synthesized. Vitamin B₁₂ deficiency leads to breakdown in homocystein remethylation and results in hyperhomocystein (Stangl *et al.* 1998). In this study, vitamin B₁₂ concentration of cattle with theileriosis was lower than healthy (control group) cattle. Serum Hcy concentrations were determined as increased in parallel with decrease in vitamin B₁₂ concentrations in cattle with Theileriosis. Changes in serum Hcy and Vitamin B₁₂ concentrations support the data of the researcher (Stangl *et al.* 1998). Increased Hcy concentrations may be result from ulcerations and disorders of heart, gastrointestinal tract and kidneys in cattle with Theileriosis.

Conclusion

As a conclusion, it is considered that Theileriosis might have basic role in producing increased concentrations of Hcy in cattle. It is concluded that, determining increased levels of Hcy may provide an insight to the researches will be performed related with Theileriosis.

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