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Levels Of Zinc, Copper And Magnesium In Sheep With Toxoplasmosis

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Summary: The aim of the present study was to investigate the effects of *Toxoplasma gondii* infection on the content of essential elements of zinc (Zn), copper (Cu), and magnesium (Mg) in sheep. Serum Zn, Cu, and Mg levels as well as wool Zn and Cu concentrations were measured in twenty sheep whose anti-*T gondii* antibodies were positive and in seventeen sero-negative healthy sheep. The average Zn concentration in serums from sero-positive sheep was significantly ($P<0.05$) lower than in their controls. However, compared to the healthy ewes the Cu concentrations in serum of sero-positive objects were recorded significantly ($P<0.001$) higher. The mean concentration of Mg in serum was higher but not significant ($P>0.05$) in sero-positive sheep than in their controls. On the other hand, the Zn and Cu concentrations of wool from diseased animals showed no significant elevations.

Key words: *Toxoplasma gondii*, zinc, copper, magnesium, sheep.

Toxoplazmozis'li Koyunlarda Çinko, Bakır ve Magnezyum Düzeyleri

Özet: Bu çalışmanın amacı *Toxoplazma gondii* enfeksiyonunun koyunlarda çinko (Zn), bakır (Cu) ve magnezyum (Mg) düzeyleri üzerindeki etkisini araştırmaktır. 20 adet enfekte ve 17 sağlıklı koyunun serumlarındaki Zn, Cu ve Mg konsantrasyonları ile yünlerindeki Zn ve Cu düzeyleri ölçüldü. Serumlarında anti-*T gondii* antikorlarını taşıyan sero-pozitif hayvanların serum Zn düzeylerinde kontrol grubu ile karşılaştırıldığında istatistik olarak anlamlı bir düşüş ($P<0.05$), serum bakır konsantrasyonlarında ise istatistiksel olarak önemli artış ($P<0.001$) saptandı. Sero-pozitif hayvanların serum Mg düzeylerinde de artışlar görülmekle birlikte bu artışın istatistiksel bir anlam ifade etmediği ($P>0.05$) tespit edildi. Yün Zn ve Cu konsantrasyonlarında ise anlamlı bir farklılığın olmadığı saptandı.

Anahtar Kelimeler: *Toxaplazma gondii*, çinko, bakır, magnezyum, koyun.

Introduction

Toxoplasmosis is a world-wide zoonosis of increasing concerning both human and veterinary medicine. *Toxoplasma gondii* is an obligate intracellular parasite living in many animal species and humans[3]. The infection sometimes causes disease and severe symptoms particularly in humans. It is an important cause abortion and perinatal mortality in sheep[3,6].

The definitive hosts of the parasite are the domestic cat and other felines, sexual cycle occurring only in these species[8]. The disease can be transmitted by ingestion of oocyst (in cat feces) or bradyzoites (in raw or undercooked)[17,25].

In humans infected with *T. gondii* disease is asymptomatic. However under some conditions, toxoplasmosis can cause serious pathology, including blindness, pneumonia, hepatitis and severe neurological disorders in patients with human immunodeficiency virus (HIV). In HIV-

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infected patients, infection with toxoplasma usually develops as a brain infection[9,22,25].

The major and trace elements play important roles in biological systems by participation in the structures or as active sites of metalloenzymes. An imbalance of mineral levels either by excess or deficiency causes alterations in respective serum levels. Many pathological conditions such as bronchitis, pneumonia, psoriasis, rheumatic heart failure, hemolytic anemia and other infectious diseases result in alterations of the trace elements levels in blood and other tissues[7].

Zinc (Zn) occurs as an important trace metal in all living organisms and all tissues. The systematic availability of Zn in tissues is highly influenced by the balance of the anabolic process regulating the renewal of soft and skeletal tissues[16]. Zn is also an essential trace element for immune function that plays a role immune response against parasites[26].

The element copper (Cu) is an integral part of cytochrome[10]. It is required for the activity of enzymes associated with Fe metabolism, elastin and collagen formation, melanin formation and integrity of the nervous system and also required for normal red blood cells formation[18].

Magnesium (Mg) is most abundant divalent cation in intracellular fluid. It is needed in many enzymes such as alkaline phosphatase, ATPase etc[1].

The concentration of trace elements was reported directly related to infectious diseases[7]. Therefore, we intended to study the effects of *Toxoplasma gondii* infection on the content of essential elements of Zn, Cu, and Mg in sheep.

Material and Methods

Twenty sheep with toxoplasmosis and seventeen healthy sheep were used in the present study. The infected sheep were given a full physical examination. No clinical signs had been noticed in any sheep used in this study. They were not receiving any steroid and medication at the time of sampling. All the sheep were 1 to 2 years old, female and nonpregnant. The toxoplasmosis in all animals was detected by Sabin-Feldman Dye test (DT) as described elsewhere previously[21]. Using DT all the animals in control group were negative for anti-*T. gondii* antibodies. In this test, antibody titer of 1/16 and over was considered as positive.

Blood samples were taken from the jugular vein and centrifuged at 1700 g for 5 min. The sera was collected in plastic tubes and frozen at –20˚C until needed for the determination of Zn, Cu, Mg.

The serum was diluted 20:1 with a solution of 0.1 M HCl and then analyzed for Zn and Cu by means of an atomic absorption spectrophotometer (Variant Spectra AA 220) as described previously[4].

Serum Mg was measured by Microlab 2000 (Merck) using commercially available kit (Biomedical Systems, Barcelona, Spain). The analyses were carried out according to the manufacturer's prescriptions.

Wools of at least 20 mm long were taken from each sheep and stored at 4°C until needed for analysis. The wool samples were washed four times in a solution containing 1% TritonX100. The washing procedure was repeated twice and then dried at 100˚C for 2 h. From each sample, 100 mg were weighed and incubated in a 1:5 mixture of nitric and perchloric acids for 6-7 hours at 60˚C. The resulting solution was diluted with 1% TritonX100 and the Zn and Cu levels were determined in the same manner as the serum samples.

Results

In table I, Zn, Cu and Mg levels analysis in serum are given in sero-positive sheep and in control animals. The mean levels of serum Zn were significantly ($p<0.05$) lower than in the healthy controls whereas serum Cu concentrations were significantly ($p<0.001$) higher.

When compared to the controls, an increase in serum Mg concentration was observed in sheep with toxoplasmosis but this change was statistically not significant ($p> 0.05$).

Table I. Comparison of serum zinc, copper and magnesium levels in sheep with toxoplasmosis and in control animals.

Parameter	Sheep with toxoplasmosis n=20 X ± SD	Control group n=17 X ± SD	*p*
Zinc (μg/dl)	68.65 ± 42.31	110.58 ± 53.25	< 0.05
Copper (μg/dl)	117.71 ± 26.91	80.58 ± 15.67	< 0.001
Magnesium (mg/dl)	2.38 ± 0.70	2.20 ± 0.74	> 0.05

In wool, Zn and Cu levels were found to be higher in sero-positive sheep than that of control animals, but these changes were not statistically significant (Table II).

A significant correlation between serum Zn and Cu concentrations as well as serum Zn and wool Cu levels were recorded ($p<0.05$).

Table II. Comparison of wool zinc and copper levels in sheep with toxoplasmosis and in control animals.

Parameter	Sheep with toxoplasmosis n=20 X ± SD	Control group n=17 X ± SD	*p*
Zinc (µg/g)	62.31 ± 34.66	54.93 ± 34.91	> 0.05
Copper (µg/g)	2.11 ± 1.29	2.01 ± 0.78	> 0.05

Discussion

There are two general classes of abnormalities associated with trace elements: specific deficiency from dietary inadequacies, imbalances, or a secondary to other diseases. Both kinds of abnormalities can be diagnosed by analyses of trace elements in serum or other tissues[14]. The trace element concentrations in serum samples change during infection[2,17]. These changes are part of defence strategies of the organism, induced by IL-1, IL-6, and TNF-∝[5,13,14].

There are a lot of studies about these essential trace elements in a various infectious diseases. It was claimed that low serum Zn levels was observed in parasitic diseases and also immune system was adversely affected during these infections[24].

In the present study, we showed that the serum Zn levels were significantly decreased in sheep with toxoplasmosis with respect to control group. Because Zn is a functional component of several enzyme systems such as carbonic anhydrase, carboxypeptidase a and b, phosphateses, several dehydrogenases. We can postulate that in the case of toxoplasmosis the whole organism of the animals affected adversely.

It has been reported[20] that the levels of serum Zn in sheep should be between 80-120 µg/dl. Similar to this statement our results from healthy animals were between 110.58 ± 53.25 µg/dl. Furthermore, the wool Zn values were measured between 54.93 ± 34.91 µg/g. The wool Zn values, obtained in this study, were remarkably lower than those of previous studies[15]. The levels of serum Zn in sheep with toxoplasmosis were significantly lower (68.65 ± 42.31 µg/dl) than that of healthy animals. The role of certain inflammatory products in regulation of the Zn balance has been well documented. Thus, leukocyte endogenous mediators (interleukins), released from activated phagocytic cells, causing a lowering of Zn levels, resulting from increased synthesis of metallothionine in liver and other tissues[14,23]. It has also been known that Zn is an essential trace element for immune function that plays a role in immune response against parasites[12]. In view of the fact that decreased Zn levels found in our study may be related to immune response against to *T. gondii*. On the other hand, wool Zn levels in diseased animals were higher but not significant than that of control animals. This increase in wool Zn level in diseased sheep remains to be elucidated.

The levels of Cu in serum from healthy animals were near to the reference values, whereas in comparison to previous findings the values of wool Cu remained under the normal values[11,15]. On the other hand, in our study, we found that serum Cu levels were significantly higher in the diseased sera than control group, but in wool the differences concerning Cu levels between diseased and healthy animals were not significant. Unfortunately, we were not able to determine the serum ceruplasmin the main Cu carrier in serum concentrations. The increased serum Cu may be in the form of serum ceruplasmin which formed in response to inflammation associated with the disease. Wool may be useful in screening long-term variations in trace element concentration by providing a better assessment of normal trace element concentrations[4,12]. In connection of this, we determined the Zn and Cu concentrations in wool from both diseased and healthy animals. However, in our study, there was no significantly difference with respect to Zn and Cu between healthy and diseased animals. We have not found any relation between antibody titre and trace element concentrations both in serum and wool measured in this study.

Interestingly, decreased Zn and increased Cu levels in serum from infected animals did not affect serum Mg concentrations. This finding is contrary to the observations of Yazar *et al*[27]. who reported decreased Mg concentrations in patients with chronic toxoplasmosis. In other words, according to our finding Mg-linked enzyme systems such as ATPase, alkaline phosphatase seem to be not affected by *T.gondii* in sheep.

In conclusion, sheep infected with toxoplasmosis showed decreased serum Zn levels and increased Cu concentrations. This may affect many of the enzyme systems. To the author's knowledge, there is no report yet about the serum Zn, Cu and Mg levels in sheep infected with toxoplasmosis. Decreased Zn and increased Cu levels recorded in this study is the first report about the sheep infected with toxoplasmosis.

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