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Relative changes in serum lipid-lipoprotein and trace element levels in cattle babesiosis*)

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Summary

The objective of this study was to investigate the relative changes in serum lipid, lipoprotein and trace element levels in babesiosis before and after treatment with diminazene aceturate (Berenil®). Babesiosis was determined with the help of the indirect fluorescent antibody test (IFAT). Cattle with babesiosis exhibited a significant increase in serum triglyceride and VLDL cholesterol levels compared to the control group. On the other hand, cattle with babesiosis had significantly lower total cholesterol, HDL cholesterol, LDL cholesterol, Fe, and Cu levels when compared to the control group. In conclusion, notable changes in lipid, lipoprotein and trace element levels were determined in cattle with babesiosis. Also we have determined a relationship between lipid and mineral metabolism in the bovine babesiosis.

Keywords: babesiosis, cattle, serum lipid, lipoprotein, trace element

Babesiosis is a tick-borne hemolytic disease caused by intraerythrocytic protozoan parasites of the genus *Babesia*, order *Piroplasmida*, which occurs in the tropical and subtropical regions (6). It is known that bovine babesiosis is widespread in Turkey (16). Chemotherapy is generally effective against bovine babesiosis. Diminazene aceturate (Berenil®) is widely used in the tropics for the treatment of bovine babesiosis (14).

Major changes in the concentration, composition, and metabolism of plasma lipids, and lipoproteins take place in several types of acute conditions. These alterations are part of the host response (1). Also the changes of serum iron (Fe), zinc (Zn), and copper (Cu) concentrations in many pathological conditions are well established. These trace elements take part in the synthesis of acute-phase proteins during infections (9).

Babesiosis is associated with nonspecific laboratory abnormalities, which can have diagnostic value when the disease is suspected (3). Hence, babesiosis might be associated with important abnormalities in lipid and trace element metabolism. In the present study, we aimed to determine the relationship between lipid and mineral metabolism in the bovine babesiosis by determining the changes in the levels of serum triglyceride, total cholesterol, VLDL cholesterol, LDL cholesterol, HDL cholesterol, and trace elements (Fe, Cu, Zn) during the acute phase of bovine babesiosis and 1 month after beginning treatment with diminazene aceturate.

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Material and methods

In the summer periods of 2001-2002, 40 Holstein dairy cattle over 1 year old from the villages around the Samsun city, Turkey, were used as the material for this study. Animals were divided into 2 groups: 20 healthy cows – the control group and 20 cows with clinical babesiosis – the patient group. Animals were assigned to proper groups after the examination of Giemsa stained thin blood smears and indirect fluorescent antibody assay (IFAT) (10). The cattle covered in this study showed symptoms suggestive of *Babesia* infection, including anorexia, anemia, fever, icterus, and hemoglobinuria, or showed *Babesia spp.* in blood smears and had seropositivity to *Babesia* infection based on IFAT.

Blood sampling was performed in the patient group on the day of diagnosis and 1 month after beginning treatment with 5 mg/kg (i.m.) diminazene aceturate (Berenil®, Hoechst Ltd., Frankfurt, Germany). Serum was removed by centrifugation of the blood samples at 2000 rpm for 10 min and the sera were kept in 1.5 ml microtubes in the deep freeze (–20°C) until analysis.

Serum was analyzed for triglycerides, total cholesterol, VLDL cholesterol, HDL cholesterol and LDL cholesterol. The analyses were performed on an automated analyzer

(XL-600, Erba, India) using commercial test kits (Teco Diagnostics, California, USA) and VLDL levels were calculated by the following formula: triglycerides/5. The concentrations of Fe, Cu, and Zn in serum samples were determined by atomic absorption spectrophotometry (5).

Dunnet test was used to compare serum triglyceride, total cholesterol, VLDL cholesterol, LDL cholesterol, HDL cholesterol, and trace element levels between the control and patient groups. The dependent t-test was used to compare these parameters within groups after the treatment. Data will be presented as mean ± standard deviation and a p value of less than 0.05 will be considered as significant.

Results and discussion

The antibody response to *B. bigemina*, *B. bovis*, and *B. divergens* of the patient group is summarized in table 1. Of the 20 calves sampled immediately before treatment, 60% were positive to *B. bigemina*, 15% were positive to *B. bovis*, and 25% were positive to *B. bigemina* and *B. bovis* (mix infection). All the cattle in the patient group were negative to *B. divergens*.

We determined the lipid profile which included serum triglyceride, HDL, LDL, and VLDL cholesterol levels (tab. 2) and serum Fe, Cu, Zn concentrations (tab. 3) of cattle with babesiosis in the control group and in the patient group 1 month after beginning treatment. The patient group exhibited a significant increase in serum triglyceride and VLDL cholesterol levels ($p < 0.001$) compared to the control group. The levels of serum triglyceride and VLDL cholesterol did not differ significantly from the control group after the treatment. On the other hand, the patient group had significantly lower total cholesterol, HDL cholesterol and LDL cholesterol levels compared to the control group ($p < 0.001$), and these levels remained low after the treatment.

In the present study, serum Fe and Cu concentrations in the patient group have been found significantly lower when compared to the control group ($p < 0.05$). On the other hand, the low levels of serum Fe and Cu in the patient group increased to the levels of the control group 1 month after the treatment with diminazene aceturate. In the study, there was no significance in serum Zn concentration differences between the control and the patient groups before and after treatment.

The present study confirmed and extended the results of preliminary studies (3, 4, 8, 15) that showed babesiosis might be related to important changes in lipid metabolism. We also determined the relationship between lipid and mineral metabolism in the bovine babesiosis. Plasma triglyceride concentration may increase, remain unchanged, or decrease in different types of acute conditions (2). In the present study, we determined that the serum triglyceride level of the patient group was significantly higher than the control group. High triglyceride levels in the patient group may be associated with increased hepatic production of triglycerides and a defect of triglyceride removal from circulation, since it has been demonstrated that increased levels of triglyceride in the acute phase response are a part of the host response (1).

The observed increase in VLDL cholesterol levels of the patient group in the study, may be related to increased adipose tissue lipolysis stimulating hepatic production of triglycerides and VLDL cholesterol. Furthermore, we think that the decrease in triglyceride and VLDL levels observed in the patient group after treatment, which eventually matched the values in the control group, might have been related to the destruction of the parasite as a result of cellular immunity caused by the secretion of inflammatory mediators from macrophages, as well as the parasite-killing effect of diminazene aceturate.

In the present study, the decrease in the serum total cholesterol levels of the patient group compared to the control group resulted from the phagocytic activity of

Tab. 1. Prevalence of antibodies against *B. bigemina*, *B. bovis* and *B. divergens* in the patient group as determined by IFA test

Antibody prevalence	Patient group			
	B. bigemina	*B. bovis*	*B. divergens*	Mix infection
Percent positive to *Babesia spp.*	60	15	0	25

Tab. 2. Serum lipid and lipoprotein concentrations in the control group, patients with acute Babesiosis and 1 month after treatment ($\bar{x} \pm SD$)

Parameters	Control group	Patients with acute babesiosis	1 month after treatment
Total cholesterol (mg/dl)	230.2 ± 62.5^a	114.9 ± 28.7^b	140.6 ± 26.3^b
HDL cholesterol (mg/dl)	71.27 ± 12.7^a	32.4 ± 6.1^b	42.5 ± 9.5^b
LDL cholesterol (mg/dl)	144.8 ± 52.3^a	81.5 ± 27.9^b	79.9 ± 19.8^b
VLDL cholesterol (mg/dl)	3.8 ± 1.27^a	10.2 ± 2.1^b	4.9 ± 1.9^a
Tryglicerides (mg/dl)	21.4 ± 6.7^a	45.2 ± 10.4^b	27.5 ± 9.3^a

Explanation: a, b – differences are statistically significant in groups marked with different letters in the same column ($p < 0.05$)

Tab. 3. Serum trace element concentrations in the control group, patients with acute Babesiosis and 1 month after treatment ($\bar{x} \pm SD$)

Trace elements	Control group	Patients with acute babesiosis	1 month after treatment
Fe (ppm)	242.8 ± 43.9^a	194.0 ± 46.3^b	243.2 ± 49.3^a
Cu (ppm)	155.1 ± 35.5^a	90.1 ± 37.1^b	148.7 ± 20.7^a
Zn (ppm)	103.7 ± 22.4^a	65.7 ± 19.5^a	72.7 ± 20.6^a

Explanation: a, b, c – differences are statistically significant in groups marked with different letters in the same column ($p < 0.05$)

macrophages and may have been the result of erythrocyte cell damage caused by the parasite. It has been demonstrated that copper modulates the activity of the rate controlling enzyme of the cholesterol biosynthesis 3-hydroxy-3-methylglutaryl-coenzyme A reductase (13). Therefore low copper concentrations that have been found in the patient group of the present study is related to the decrease in cholesterol levels and may have been the result of anemia that occurs in Babesiosis.

In normal conditions, LDL cholesterol transports cholesterol, phospholipids, and lipid-soluble vitamins from the liver to extra-hepatic tissues. And HDL cholesterol plays a major role in the reverse transport of cholesterol from peripheral tissues to the liver. Several types of acute conditions are associated with a marked decrease in HDL cholesterol (1). The decrease in serum LDL and HDL cholesterol levels observed in the patient group may have been related to the decrease in total cholesterol levels in the patient group. Similar changes in serum lipid profiles have also been reported in rabbits with *Trypanosoma brucei brucei* (12), and in humans with severe leptospirosis (11) and babesiosis (3).

There is no considerable evidence for a relationship between essential trace elements and tropical infections. However, charasteristic changes in trace element metabolism is reported in many parasitic diseases, such as leishmaniasis (9) and malaria (7). Anemia is a common clinical sign that occurs in Babesiosis. It has been demonstrated that anaemic animals consume body iron and copper reserves, which leads to a decrease in the levels of these two elements. Therefore the decrease in the concentrations of Fe and Cu in the patient group can be explained by the anemia occurring in acute babesiosis. Cu, Fe and Zn also take part in important enzymes of the immune cells. Since these enzymes are induced by cytokines, the alterations of serum Fe, Cu and Zn that has been found in the present study, may also depend on cytokines, especially IL-1 and TNF-α, as a part of the acute-phase response of the host.

The present study demonstrated important abnormalities in lipid and trace elements in the bovine babesiosis. Moreover, the results of our research suggest that there is a relationship between lipid and mineral metabolism in the acute babesiosis.

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