# Studies on the Effect of Iron (Fe) Preparations in Addition to Babesiosis Treatment on the Haematological and Some Mineral Levels in Sheep Naturally Infected with *Babesia ovis*

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***SUMMARY***

*In the present study, in addition to rutine babesiosis treatment the impact of oral iron application was investigated. For this purpose; 14 Akkaraman sheep infected with babesiosis and 7 healthy Akkaraman (as control) were used as animal material. The infected sheep were divided into two equal groups as group I and group II. Animal in group I received 50 mg/kg iron preparation (Ferrum Forte, Hausman® Aİ) in addition to diminazen diaceturate (Berenil®, Topkim) application. On the other hand, animal in group II received only diminazen diaceturate . Erytrocyte, haemoglobin, haematocrit values of both groups (group I, group II) were lower than control group before treatment. On the other hand, cupper and iron values were higher than control group in both groups before treatment. 1. and 7. days after treatment statistical differences were disappeared in terms of examined haematological parameters. Although it wasn't significant, cupper and iron values obtained from group I increased a day after treatment compared to the values obtained before treatment. Furthermore, 7 days after treatment, although it wasn't significant, cupper and iron values in both group decreased. As a result; in the treatment of babesiosis, in addition to diminazen diaceturate application using iron preparation had no contribution on the treatment of babesiosis.*

***Key words**: Sheep, Babesia ovis, Iron, Mineral substance*

***Doğal olarak Babesia ovis'le Enfekte Koyunlarda Sağaltıma Ek Olarak Demir ( Fe) Preparatı Uygulamasının Hematolojik Parametreler ve Bazı Mineral Madde Düzeyleri Üzerine Etkisinin Araştırılması***

***ÖZET***

*Bu araştırmada rutin babesiosis tedavisine ek olarak oral demir uygulamasının tedaviye etkinliği araştırıldı. Bu amaçla 14 adet B. ovis'le enfekte ve 7 adet sağlıklı (kontrol grubu) olmak üzere 21 Akkaraman ırkı koyun kullanıldı. Babesiosisli koyunlar tedavi öncesi bir örnek iki gruba ayrıldı. Bir gruba (I. Grup) diminazenaseturat'a ek olarak 50 mg/kg dozunda oral demir, diğer gruba (II. Grup) ise sadece diminazenaseturat uygulandı. Her iki grubun sağaltım öncesi eritrosit, hemoglobin ve hematokrit değerlerinin kontrol grubuna göre önemli derecede düşük, demir ve bakır değerlerinin ise yüksek olduğu belirlendi. İncelenen hematolojik parametreler açısından tedavi sonrası (1. ve 7. günler) tedavi öncesine göre her iki grupta istatistiki olarak önem arz etmedi. Tedavi sonrası I. grup 1. günde demir ve bakır düzeyleri istatistiki önem arz etmemesine rağmen artış gösterdi. Tedavi sonrası 7. günde tedavi öncesine göre demir ve bakır değerleri her iki grupta istatistiki önem arz etmemesine rağmen azaldı. Sonuç olarak; babesiosis tedavisinde diminazenaseturat'a ilave olarak demir preparatı uygulamasının tedaviye katkı sağlamadığı kanısına varıldı.*

***Anahtar kelimeler:** Koyun, Babesia ovis, Demir, Mineral madde.*

## INTRODUCTION

Babesiosis is a protozoan disease, which is generally characterized with high fever, anorexia, weight loss, ruminal atony, dyspnoea and jaundice, of sheep, goat, cattle, horse, dog, and cats. The vector places itself in animal's erythrocytes and Babesia forms can vary as pear-shaped, round and elongated. The most common species that causes infection on sheep are *B. ovis* and *B. motasi,* which are primarily transmitted by *Rhipicephalis bursa* ticks (3, 7, 8, 11, 15). The mucous membranes are first hyperaemic, but at the later stages, they become icteric and the colour changes to the pallor of anemia. Primary cause of anemia is due to intravascular haemolysis and rate of destruction of erythrocytes and capacity of erythropoiesis are the main denominators of the occurrence and intensity of the anemia (6, 9, 14). It has been demonstrated that *B. ovis* is less pathogenic than *B. motasi* for sheep infection and causes relatively moderate haemolytic anemia (3, 8). While anaemia is very common for all infected animals haemoglobinuria may not be observed with the animals infected with *B. ovis* (3, 7, 8, 11 ). The infection can be detected on May Grunwald + Giemsa-stained thin blood smears through identification of the vectors in erythrocytes via microscopic examination (3, 7, 10, 11, 15, 14).

The most commonly used compounds for the treatment are quinine, diamidin and other synthetic urine extractions. In addition to these, several studies have demonstrated that glucocorticoids, vitamin $B_{12}$ and iron preparation may be used for treatment (3, 8). Iron is most commonly used element for treatment of anemia, malnutrition and can be used in addition with other drugs for the treatment of several diseases. Some researches stated that for anemic animals organic and inorganic iron salt could be orally administered and the recommended dose for each sheep could be between 0.5 and 2 grams (8).

The aim of this study was to ascertain the effect of iron treatment, which was used along with diminazene

diaceturate, on haematological parameters and several mineral levels and to identify the contribution of the supplementary iron treatment, if any, on the treatment of naturally *Babesia ovis* infected sheep.

## MATERIAL and METHOD

The study was carried on total 21 Akkaraman sheep (between 1 and 3 years of age) of which 14 sheep were among the ones which were identified as a result of both clinical and laboratory researches as infected with Babesiosis between June and August of 2002, and of 7 were health sheep gathered from dairy areas in Özalp District of Van Province. Several blood smears obtained from each Babesiosis infected sheep and these May Grunwald + Giemsa-stained blood smears were examined under microscope and it was observed that *B. ovis* was the primary cause for the infection. Blood samples at 0, 1st and 7th days to measure haematological parameters and plasma mineral substance levels were obtained from Vena jugulars and put into Lithium heparin tubes via using disposable cannulas. In addition, further blood smears were obtained at 7th day following the treatment from each animal to detect *Babesia* vector.

Total 14 infected sheep was divided into two equal groups as Group-1 (n=7) and Group-2 (n=7) once blood samples were gathered prior to the treatment (at 0 day). While Group-1 was orally administered with 50mg/kg iron (Fe), (Ferrum Forte, hausman ® Aİ) along with 3,5 mg/kg diminazene diaceturate (Berenil ®) 7% solution administered via intramuscular injection, only 3,5 mg/kg diminazene diaceturate (Berenil ®) 7% solution was administered to Group-s via intramuscular injection.

To ascertain occurrence of the anaemia and its intensity several measurements were done on whole blood samples to determine haemoglobin concentration (Hb) via colorimetric spectrophotometer; haematocrite value (Htc) through microhaematocrite method; erythrocytes count (RBC) with Hayem solution and leucocytes count (WBC) with Leucocytes solution via Thoma slide. For certain mineral levels in the plasma, on the other hand, were measured via Atomic Absorption Spectrophotometer (UNİCAM 929).

All numerical data was statistically evaluated via paired-samples test through SPSS statistic software.

## FINDINGS

Prior to the treatment, examinations on all infected animals demonstrated that all animals presented ticks and high fever, anorexia, and tachycardia were the common diagnosis; and conjunctiva and mucous membranes presented colour change (ranging from hyperaemic, icteric and anaemia). Besides 3 of 7 sheep in Group-1 and 2 of in Group-2 were identified as having haemoglobinuria. It has been ascertained that all clinical diagnosis was recovered after a 7-day treatment.

Table 1. Various Haematological and Some Mineral Levels

| Parameters | Control (n=7) $\overline{X}$ ±SE (X min – X max) | 0. Day (n=14) $\overline{X}$ ±SE (X min – X max) | Group-1 (n=7) Day 1 $\overline{X}$ ±SE (X min – X max) | Group-1 (n=7) Day 7 $\overline{X}$ ±SE (X min – X max) | Group-2 (n=7) Day 1 $\overline{X}$ ±SE (X min – X max) | Group-2 (n=7) Day 7 $\overline{X}$ ±SE (X min – X max) |
|---|---|---|---|---|---|---|
| RBC ($10^3/mm^3$) | 11716±71[a] (9500-14100) | 68885±62[b] (4500-9200) | 7942±65[c] (6100-11000) | 8657±10[d] (5100-12000) | 7680±88[c] (6200-11000) | 9140±12 (6800-14000) |
| WBC ($mm^3$) | 7633±68 (6100-10200) | 7500±14 (5100-12500) | 10385±11 (6500-14300) | 8242±11 (4500-12500) | 5760±71 (4000-7500) | 6720±75 (4100-8300) |
| Htc (%) | 34±3 [a] (27-42) | 20±2[b] (12-30) | 25±2.7[c] (18-40) | 29±5.0 (13-48) | 24±3.8 (17-39) | 31±4.1 (20-40) |
| Hb (gr/dl) | 12.2±0.7 [a] (9.8-14.4) | 7.3±0.7[c] (5.0-11.0) | 8.4±0.8[c] (6.1-13.0) | 9.3±1.3 (5.0-14.0) | 8.16±1.2[d] (6.1-12.9) | 10.5±1.2 (7.1-13.1) |
| Fe (mg/L) | 3.10±0.24 [a] (2.6-4.2) | 5.29±0.62[d] (3.1-7.8) | 6.47±0.87[c] (3.5-10.2) | 4.31±0.41[d] (2.7-5.6) | 4.60±1.02 (2.7-8.5) | 4.82±0.90 (2.3-7.7) |
| Cu (mg/L) | 0.898±0.15 [a] (0.396-1.380) | 1.550±0.10[c] (1.212-1.974) | 1.608±0.12[c] (1.242-1.929) | 1.496±0.25[c] (1.194-1.920) | 1.596±0.13[c] (1.194-1.920) | 1.199±0.11 (0.924-1.530) |
| Ca (mg/L) | 5.33±0.22 (3.80-6.19) | 5.54±1.01 (2.40-7.40) | 6.04±1.09 (2.54-7.70) | 4.37±0.61 (2.81-8.42) | 5.88±1.21 (2.52-8.72) | 6.04±1.33 (2.82-9.32) |
| Zn (mg/L) | 1.727±0.18 (1.428-1.926) | 1.754±0.26 (1.464-2.310) | 1.718±0.15 (1.512-1.812) | 1.682±0.20 (1.500-1.728) | 1.645±0.18 (1.386-1.940) | 1.630±0.14 (1.428-1.878) |

a, b, c, d: The different letters in the same row represents the statistical significance between groups ab: $p<0.001$, ac: $p<0.01$, ad: $p<0.05$.

## DISCUSSION and CONCLUSION

It has been informed that iron preparations could be used for Babesiosis treatment as well as babesidal drugs, antibiotic with larger spectrums, and vitamin B (3, 8, 10 ). This study examined the contribution of iron administration accompanied with diminazene diaceturate in treating naturally *Babesia ovis* infected sheep.

The severeness of the clinical symptoms that were observed with animals infected with Babesiosis very

much depends on the characteristic of the virus afflicted the animal, the concentration of the causative vector in blood and the strength of the animal's immune system (3, 8, 14). The clinical symptoms appear as the animal has a high fever with temperatures reaching > 40.0 C°. There is anorexia, ruminal atony, tachycardia, and haemoglobinuria (1, 4, 8, 14, 10). The results of this study showed that the clinical diagnosis observed with the infected animals were in conformity with the results of other studies carried out by different researches concerning animals infected with Babesiosis (1, 4, 8, 14, 10).

Haematological findings that were observed with domestic animals in connection with blood parasite infections are very essential. In this study it was demonstrated that value of haematological parameters (RBC, Htc, Hb) of the group, compromising infected animals, were relatively lower compared to control group parameters prior to the treatment (at Day 0). This might have been aroused from that clinical Babesiosis might have restricted erythropoietic activity in bone marrow. This was also confirmed by the findings of other studies carried out by other researchers (10, 14, 15).

Haematological parameters identified by the study conducted by Pandey and Misra (10), concerning with the administration of iron as well as diminazene diaceturate in treating the cattle infected by Babesiosis were as follows: RBC 3.44±0.23 $10^6/mm^3$, WBC 1285±439.12 $mm^3$, Htc 17±0.68 (%), Hb 6.48±0.34 (% ) prior to the treatment and RBC 5.68±0.16 $10^6/mm^3$, WBC 8492±238.88 $mm^3$, Htc 26.14±0.59 %, Hb 9.69±0.16 % post-treatment. Based on these findings, the observed Haematological parameters in post-treatment period represented statistical significance compared to parameters observed prior to treatment. These findings of the researches go parallel with the findings observed with the Group-1 at $7^{th}$ day post-treatment in the study where iron as well as diminazene diaceturate were administered in treatment.

During the study conducted by Voyvoda and his colleagues (14) 0.5 % Quinurium sulphate solution treatment was applied to the naturally *B. ovis* infected sheep. Haematological parameters found in this study were as follows. Htc 19.07±0.78 (%), Hb 7.00±0.30 (%) prior to the treatment and Htc 22.27±0.81 (%), Hb 8.04±0.29 (%) post treatment. These findings of the researches go parallel with the findings observed with the Group-2 at $7^{th}$ day post-treatment in the study where only diminazene diaceturate was administered in treatment.

Iron and copper are two elements that can be profoundly observed in the cells of plants and animals as well as in soil and water. It has been demonstrated that copper is essential to use of iron in haemoglobin production thus lack of copper results in intervention in haematopoiesis, which leads to anaemia (12, 5, 13). It has been demonstrated that anaemic animals would start consuming bodily iron and copper reserves, which leads to decrease in levels of these two elements is blood serums (4, 12, 13, 16).

With this study on the sheep infected with Babesiosis, we have found out that average blood serum iron level was 5.29±0.62 mg/L and copper level was 1.550±0.10 mg/L (Day 0). These levels were significantly higher than those of control group were. While these findings go parallel with the findings of the study conducted by Voyvoda and his colleagues (14) they, on the other hand, differs from the findings of Biçek and his colleagues (4). Having observed that the blood serum iron and copper levels for the animals infected with Babesiosis were relatively higher and haematological parameters lower compared to the control group values we concluded that this might have been as a result of the intravascular haemolyses occurred on these animals infected by Babesiosis.

The present study demonstrated that the iron levels for the group-1 animals treated with iron as well as diminazene diaceturate were not statistically significant yet higher compared to prior treatment ($1^{st}$ day) period and group-2 values. We concluded that this might have been due to iron treatment.

Pandey and his colleague (10) have demonstrated that both iron and copper levels prior to treatment significantly decreased after the treatment. Voyvoda and his colleague (14) have noticed that iron level after treatment was decreased compared to the levels measured before treatment. Whereas the findings of this study confirm the findings of the study of Pandey and his colleague (10) they, on the other hand, differ from the findings of Voyvoda (14) and his colleague. Regarding copper levels findings confirms with both of above-mentioned studies. During treatment period due to decrease in intravascular haemolyses and increase in erythropoietic activity the need for iron and copper will increase. Therefore the decrease in these levels may be explained as a result of this process occurs during the treatment period.

Regarding zinc and calcium levels, it has been ascertained that the findings related with the animals infected with Babesiosis (group 1 and 2) represent no statistical significance whatsoever compared to control groups values. While results showing the calcium levels were in line with the findings of other researches (2, 10) we, on the other hand, found no material related to the zinc levels in the literature.

In sum, we ascertained that RBC, Hb and Htc values measured in the animals infected with Babesiosis were relatively lower compared to the control group's values. We also observed that whilst the iron and copper levels were significantly higher there was no statistical difference between two groups (group 1 and group 2) after treatment. We suggest that the increase in iron level might have been resulted from intravascular haemolysis and that the changes observed in copper levels might have been related with the iron levels. Based on these above-mentioned findings we concluded that administration of iron in the treatment of animals infected with Babesiosis had no contribution to the routine treatment.

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