The Effect of Classical Theileriosis Treatment on Thyroid Hormone Levels in Cattle Naturally Infected with Theileria annulata

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ABSTRACT
The aim of this study is investigated the effect of classical theileriosis treatment on thyroid hormone levels [triiodothyronine (T3), thyroxine (T4) and thyrotropin (TSH)] when applied to cattle naturally infected with Theileria annulata. The material of the study encompasses 10 cattle of different races and ages from two strains (male and female) which are diagnosed with theileriosis (patient group) and 10 cattle which are healthy according to clinical examination (control group), a total of 20 cattle. Blood samples of patient group were taken two times as pre-treatment and post-treatment (a month later), the blood samples of the control group were taken once. In haematological examinations, the number of erythrocytes and leukocytes was determined using Thoma lamina; the hematocryte value was determined using the capillary tube and the amount of haemoglobin was determined according to Sahli’s method. The free and total T3, T4 and TSH concentrations were analyzed in autoanalyzer according to the electrochemiluminescence immunological test (ECLIA) method. It was detected that mean values of free T3, free T4, total T3 and total T4 of thyroid hormones were significantly lower than the values of the control group in pre-treatment period (p<0.001, p<0.01, p<0.001 and p<0.01, respectively), mean value of the TSH increased (p<0.05). In post-treatment analysis, there was not statistical difference in all thyroid hormones except for total T4 concentration. It was established that although there was increase in total T4 level when compared with pre-treatment values, the difference between control group and the groups was low on the level of p<0.05. Consequently, it was determined that, besides the classical treatment, no additional treatment is required to establish normal thyroid hormone levels, although the thyroid hormones were seen to decrease in cattle infected with T. annulata.

Key words: Theileria annulata, tropical theileriosis, thyroid hormones, cattle, treatment

INTRODUCTION
Theileria annulata is a tick-transmitted protozoan affecting cattle and is the causative agent of tropical theileriosis (Ahmed and Mehlhorn, 1999; Keles et al., 2001). Tropical theileriosis is a progressive lymphoproliferative disease of cattle caused (Gul et al., 1991; Keles et al., 2001; Prestoni et al., 2002; Rezaei and Dalie-Naghadeh, 2006). Theileria annulata is seen in tropical and subtropical regions (Gul, 2006) and progresses together with severe anemia (Altug et al., 2008; Gul et al., 1991; Issi and Gul, 2001; Omer et al., 2002; Saber et al., 2008).

It was declared in the literatures (Altug et al., 2008; Gul, 2006; Gul et al., 1991; Keles et al., 2001) that buparvaquone (2.5 mg kg⁻¹ a single dose, IM) and oxytetracycline (10 mg kg⁻¹, IM)
should be used at the treatment. Additionally, in animals with severe anemia 5% dextrose and vitamin B₁₂ should be used as supportive treatment (Altug et al., 2008; Keles et al., 2001).

It was reported that thyroid hormones are affected in cases of tropical theileriosis caused by Theileria annulata (Badiei and Jaber, 2002; Garg et al., 2001; Sangwan et al., 2002). It is stated that thyroid hormones, which affect growth, development, energy and efficiency metabolisms necessary for the development and normal functioning of many cells (Guyton, 1986; Sanli, 1999; Turgut, 2000), are closely associated with the regulation of oxygen consumption (Guyton, 1986; Sawhney and Malhotra, 1990; Sanli, 1999). The mechanism of hypoxia, which affects the secretion of thyroid hormone, is not yet fully understood (Sawhney and Malhotra, 1990). In the literature (Galton, 1972; Hess et al., 2002; Surks, 1990), it is reported that the thyroid metabolism may deteriorate as a result of the decrease in hypoxia and oxygen transport due to anaemia.

Total T₃ and total T₄ levels are 2.20-2.60 ve 79.70-91.80 nmol L⁻¹ in calves (Altintas et al., 1995); 0.030-3.53 ve 43.7-128.7 nmol L⁻¹ in heifers (Baysu and Dundar, 1986) 0.60-2.62 ve 49.17-67.06 nmol L⁻¹ in cattle (Blum et al., 1988), respectively.

The present study examined the effect of classical theileriosis treatment on thyroid hormone levels (TSH, free and total T₃ and T₄) when applied to cattle naturally infected with Theileria annulata.

MATERIALS AND METHODS

The research material consisted of 20 cattle from both sexes (10 male and 10 female) and of various breeds (10 Swiss Brown, 6 Holstein and 4 Simmentals) and ages (1–3 years). All animals were from Elazig district, Turkey and were subjected to similar management conditions during the period from April 2009 to September 2009. The patient group (n = 10) had been diagnosed as being naturally infected with tropical theileriosis. The control group (n = 10) had similar characteristics to the patient group, but were shown to be healthy in clinical examinations. The patient group was brought to the Firat University Faculty of Veterinary Animal Hospital Internal Diseases Clinic for examination and treatment. Blood samples were taken from the patient group before the treatment (Before Treatment) and 1 month after the treatment (After Treatment); blood samples were taken from the animals in the control group only once.

Thin blood smears were extracted from the ear tips of all the animals in accordance with the relevant guidance. Samples were coloured with Giemsa and analyzed under a microscope. Having observed the pyroplasmic forms of the T. annulata factors in erythrocytes, tropical theileriosis was diagnosed (Altug et al., 2008; Azizi et al., 2008; Gul, 2006; Omer et al., 2002).

Blood samples which included a sufficient amount of EDTA with appropriate characteristics were taken from the V. jugularis of all the animals. Samples were taken into sterilized glass tubes for biochemical analysis by means of haematological examination. Serum samples were kept at -20°C until free and total T₃, T₄ and TSH levels were analyzed.

In haematological examinations, the number of erythrocytes and leukocytes was determined using Thoma lamina; the hematocryte value was determined using the capillary tube and the amount of haemoglobin was determined according to Sahli’s method (Schalm et al., 1983). The free and total T₃, T₄ and TSH concentrations were analyzed in a Cobas 6000 autoanalyzer according to the electrochemiluminescence immunological test (ECLIA) method.

A single dose of 2.5 mg kg⁻¹ IM buparvaquone (Tailorol; Provet) was administered to the animals which were diagnosed with theileriosis (Before treatment group) and a dose of 10 mg kg⁻¹
IM oxytetracycline (Primamycin LA; Pfizer) was administered each day for 5 days. In addition, 5% dextrose (Dextrosol; Vilsan) and vitamin B<sub>12</sub> (Dodeks; Vetas) were administered to the animals with severe anaemia.

Statistical analysis of the data was performed using SPSS (SPSS Chicago, IL, USA, Version 13 for Windows). The results are shown as arithmetical average and standard error. The t-test was utilized in determining the materiality between the groups.

RESULTS

Examination of the patient group prior to treatment detected anorexia, cough, growth in superficial lymph nodes and petechial blood blisters in conjunctivas and it was shown that mucosas were clearly anaemic or slightly icteric. Post-treatment examination showed that the appetite of all the animals had returned to normal, although some had slight anaemia.

The general clinical (body temperature, heart and respiration frequency and rumen movements) and haematological findings (the number of erythrocytes, total number of leukocytes, hematocyte value and the amount of haemoglobin); the arithmetic mean of thyroid hormone levels (Free T<sub>3</sub> and T<sub>4</sub>, total T<sub>3</sub> and T<sub>4</sub>, TSH); minimum and maximum values and the importance of the difference between the groups are given in Table 1-3.

When the Table 1 is examined, while body temperature (p<0.001), heart rate (p<0.01) and breathing rate (p<0.01) are increasing in patient group before treatment according to patient group, rumen movement is decreasing. After treatment while statistical importance among groups was seen in body temperature (p<0.01), heart rate (p<0.01), differences among groups were not seen in breathing rate and rumen movement.

In Table 2 given hematological parameters, all parameters (Total leukocyte count, Erythrocyte count, packed cell volume and Haemoglobin value) in before treatment group decreased and all parameters in after treatment group increased but only total leukocyte count did not altered among the groups.

Table 1: Arithmetic averages of the general clinical examination findings of control and patient group (before treatment and after treatment) animals and the significance of the difference between the groups

<table>
<thead>
<tr>
<th>Clinical findings</th>
<th>Control group</th>
<th>Before treatment</th>
<th>p-value</th>
<th>After treatment</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body temperature (°C)</td>
<td>38.0±0.10 (37.5-38.6)</td>
<td>40.4±0.23 (39.5-41.7)</td>
<td>&lt;0.001</td>
<td>38.5±0.18 (37.9-39.5)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Heart rate (min)</td>
<td>72.6±1.23 (68-78)</td>
<td>83.6±3.90 (64-104)</td>
<td>&lt;0.01</td>
<td>68.0±0.84 (64-72)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Breathing rate (min)</td>
<td>27.8±0.96 (24-32)</td>
<td>35.8±1.96 (24-44)</td>
<td>&lt;0.01</td>
<td>27.8±2.35 (24-32)</td>
<td>NS</td>
</tr>
<tr>
<td>Rumen movement (5 min)</td>
<td>8.8±0.13 (8-9)</td>
<td>3.8±0.47 (2-6)</td>
<td>&lt;0.001</td>
<td>8.4±0.52 (7-10)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Results are expressed as Mean±SEM. Extreme values are given into parenthesis

Table 2: Arithmetic averages of the hematological examination findings of control and patient group (before treatment and after treatment) animals and the significance of the difference between the groups

<table>
<thead>
<tr>
<th>Haematological parameters</th>
<th>Control group</th>
<th>Before treatment</th>
<th>p-value</th>
<th>After treatment</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total leukocyte count (x10&lt;sup&gt;9&lt;/sup&gt; L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>8.4±0.23 (7.6-9.8)</td>
<td>4.3±0.58 (2.2-7.6)</td>
<td>&lt;0.001</td>
<td>7.6±0.47 (6.6-9.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Erythrocyte count (x10&lt;sup&gt;12&lt;/sup&gt; L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>7.0±0.25 (6.2-8.31)</td>
<td>4.0±0.40 (1.8-6.74)</td>
<td>&lt;0.001</td>
<td>5.7±0.17 (4.8-6.43)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Packed cell volume (%)</td>
<td>31.6±0.95 (28-38)</td>
<td>18.1±1.68 (10-24)</td>
<td>&lt;0.001</td>
<td>25.8±0.82 (23-30)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Haemoglobin (g dL&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>10.8±1.27 (10.0-13.0)</td>
<td>8.7±1.67 (8.8-10.2)</td>
<td>&lt;0.001</td>
<td>8.9±0.19 (8.2-10.0)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Results are expressed as Mean±SEM. Extreme values are given into parenthesis
Table 3: Arithmetic averages of the thyroid hormones of control and patient group (before treatment and after treatment) animals and the significance of the difference between the groups

<table>
<thead>
<tr>
<th>Thyroid hormones</th>
<th>Control group</th>
<th>Patient group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>p-value</td>
</tr>
<tr>
<td>Free T₄ (pmol L⁻¹)</td>
<td>5.85±0.39 (3.83-7.46)</td>
<td>3.74±0.34 (1.55-5.09)</td>
</tr>
<tr>
<td>Free T₃ (pmol L⁻¹)</td>
<td>15.3±2.0 (11.13-19.44)</td>
<td>11.6±0.56 (8.52-17.56)</td>
</tr>
<tr>
<td>Total T₃ (ng dL⁻¹)</td>
<td>2.4±0.12 (1.68-2.88)</td>
<td>1.4±0.17 (0.67-2.27)</td>
</tr>
<tr>
<td>Total T₄ (μg dL⁻¹)</td>
<td>98.3±5.13 (82.86-128.1)</td>
<td>69.7±7.91 (42.11-111.5)</td>
</tr>
<tr>
<td>TSH (μIU mL⁻¹)</td>
<td>0.009±0.000 (0.005)</td>
<td>0.005±0.000 (0.005-0.007)</td>
</tr>
</tbody>
</table>

Results are expressed as Means±SEM. Extreme values are given into parenthesis.

Troid hormone levels except TSH in before treatment and after treatment groups in Table 3 decreased according to control group.

DISCUSSION

Tropical theileriosis is a protozoan disease of blood and lymph tissues. It can cause great economic losses due to the mortality rate reaching 100% as a result of severe anaemia, high fever, anorexia, inactivity, decrease in efficiency, rumen movements and decrease in rumination, respiratory distress, paleness in mucosa and conjunctivae, petechia, echymotic blood blisters and secondary infections, especially in cultivated cattle (Altug et al., 2008; Gul, 2006; Gul et al., 1991; Issi and Gul, 2001; Omer et al., 2002).

Tropical theileriosis was diagnosed upon finding the pyroplasmic forms of T. annulata in erythrocytes in all the animals in the patient group. This was consistent with sources (Altug et al., 2008; Gul, 2006; Issi and Gul, 2001; Omer et al., 2002) that suggest that definitive diagnosis can be made when T. annulata factors are found in the blood smear. It was observed that all the clinical (Table 1) and haematological findings (Table 2) obtained in the patient group prior to treatment are consistent with the symptoms described for tropical theileriosis (Altug et al., 2008; Gul, 2006; Gul et al., 1991; Issi and Gul, 2001; Omer et al., 2002). As can be seen from Table 1 and 2, the average values of the clinical and haematological parameters after treatment in both control and patient groups are within the normal limits stated for healthy animals (Issi and Gul, 2001; Smith, 2009).

All the animals in the patient group were treated in line with recommendations in the literature (Altug et al., 2008; Gul, 2006; Gul et al., 1991) on the specific use of buparvaquon and oxytetracycline and that supportive treatment should be provided to animals with severe anaemia.

It is stated that the serum concentrations of thyroid hormones should be measured in the assessment of the functional status of the thyroid gland (Turgut, 2000). It is also stated that most of the secreted hormones (over 90%) are generally T₄ and the rest are T₃; and 0.03% of T₄ and 0.2 to 0.5% of T₃ are free in the plasma (Sanli, 1999). There are different reference values within the literature regarding the physiological values of serum T₃ and T₄ levels in cattle (Altintas and Fidanci, 1993; Altintas et al., 1995; Baysu and Dundar, 1986; Bellman et al., 2004; Blum et al., 1983; Sezer and Sahinduran, 2004). In the cattle in the present control group, it was detected that the levels of free T₃, free T₄, total T₃, total T₄ and TSH were 5.85±0.39 pmol L⁻¹, 15.3±0.85 pmol L⁻¹, 2.4±0.12 ng dL⁻¹, 98.3±5.13 μg dL⁻¹ and 0.005 μIU/mL, respectively.

Badiei and Jaber (2002) stated that the T₃ and T₄ levels in cattle naturally infected with T. annulata were 14.2 ng dL⁻¹ and 2.25 μg dL⁻¹, respectively, which are significantly lower than
those values detected in healthy cattle (p<0.05). Sangwan et al. (2002) and Garg et al. (2001) reported that thyroid hormones decrease in tropical theileriosis. In the present study, free T₃, free T₄, total T₃, total T₄ and TSH levels, while a statistically significant difference was found between the average values of the control group and the pre-treatment group (p<0.001, p<0.01, p<0.001, p<0.01 and p<0.05, respectively), no statistically significant different was found in the post-treatment analysis in the other parameters except for the total T₄ level (p<0.05). Although, no treatment was applied to the animals in the patient group in order to improve thyroid function, it was observed that there was no statistically significant difference between the average parameter values of the control group and the patient group following treatment and these values were within the physiological limits stated for healthy animals (Sezer and Sahinduran, 2004).

Among the pre-treatment hematologic examination findings of the animals in the patients group there was a significant difference between the groups (p<0.001) in terms of number erythrocytes, haematocrit value and the amount of haemoglobin, which point out anemia. These findings support the view that the most important finding of tropical theileriosis is anaemia. These results support the conclusions of previous studies (Altug et al., 2008; Gul et al., 1991; Issi and Gul, 2001; Omer et al., 2002), which reported that the most important effect of tropical theileriosis is anaemia. In accordance with reports that thyroid function can deteriorate in anaemia due to reduced hypoxia and oxygen transport (Galton, 1972; Hess et al., 2002; Surks, 1966), it is thought that the reduced thyroid hormone levels detected in the present study are a result of reduced oxygen transport due to anaemia.

Consequently, it was determined that, besides the classical treatment, no additional treatment is required to establish normal thyroid hormone levels, although the thyroid hormones were seen to decrease in cattle infected with T. annulata.

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