Research Article

Haematological and Coagulation Profiles during Severe Tropical Theileriosis in Cattle

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Abstract: This study was conducted to measure selected haematological parameters in Holstein cattle naturally infected with *Theileria annulata*. Haematological analysis indicated significant decreases in red blood cell count, haematocrit value, haemoglobin amount, mean corpuscular haemoglobin concentration, and white blood cell, lymphocyte, neutrophil, monocyte, eosinophil, and basophil counts. On the other hand, significant increases were seen in mean corpuscular volume and marked reticulocytosis in infected animals compared to the animals in the control (uninfected) group. In the coagulation profile, activated partial thromboplastin time and prothrombin time were significantly prolonged, and platelet counts were significantly less in the infected group compared to those in the control group. Fibrinogen concentration was slightly higher in the infected group than that in the control group. These observations revealed that *T. annulata* infection in cattle is associated with marked changes in haematological and coagulation parameters.

Key Words: *Theileria annulata*, haematology, coagulation, cattle

Şiddetli Tropikal Theileriosisli Sığırlarda Hematolojikal ve Koagulasyon Profilleri

Özet: Bu araştırma, doğal olarak *Theileria annulata* ile infekte olan Holstein sığırlarda bazı hematolojik ve hemostatik parametrelerin belirlenmesi amacıyla yapıldı. Kontrol grubu ile karşılaştırıldığında, enfekte grupta alyuvar sayısı, hematokrit değer, hemoglobin mikratı, ortalama alyuvar hemoglobin konsantrasyonu, akyuvar sayısı, lenfosit, nötrofil, monosit, eozinofil ve bazofil sayıları önemli düşüşler gösterirken belirgin bir retikülositozis ile birlikte ortalama alyuvar hacminde ciddi artışlar görüldü. Kontrol grubu ile karşılaştırıldığında, enfekte grupda aktive edilmiş parsiyel tromboplastin zamanı ve protrombin zamanında önemli derecede uzama, trombosit sayısında önemli oranda düşme, fibrinojen konsantrasyonunda ise hafif bir yükselme bulundu. Sonuç olarak; Tropikal theileriosisin, sığırların hematolojik ve hemostatik profilinde önemli düzeyde değişikliklere sebep olduğu belirlendi.

Anahtar Sözcükler: *Theileria annulata*, hematoloji, koagulasyon, sığır

Introduction

Tropical theileriosis, a tick-borne haemoprotozoan disease caused by *Theileria annulata* and transmitted by *Hyalomma* spp., is one of the most devastating blood parasites affecting cattle (1). The prevalence, morbidity, and mortality of tropical theileriosis are considerably high (2,3). It is estimated that 250 million cattle in many countries, including the southern Mediterranean countries, Turkey, India, and China, are at risk from the disease, which causes serious economic loss through bovine mortality and lost productivity (4). In their mammalian hosts, the parasites have a complex life cycle. Infection is initiated by the transformation of macroschizont-infected cells in the lymph nodes draining the site of inoculation of sporozoites by ticks. The cattle-infective form of the parasite is the sporozoite transforming into schizonts in white blood cells of the mononuclear lineage. The schizont undergoes further differentiation to merozoites, which are released upon lysis of the infected cells. Once released from host cells, the merozoites enter erythrocytes. This is followed by the development of piroplasms in erythrocytes and the parasite becomes infective for the vector. (5).

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Haematological and coagulation parameters are a useful tool for diagnosis, prognosis, and therapy. These parameters provide highly valuable information about the severity of infection; therefore, in the present study, changes in these parameters were investigated to further understand the pathogenesis of tropical theileriosis.

Materials and Methods

Field Study Area and Animals

The present investigation was carried out during the disease seasons from May 2004 to July 2005 in villages within 10-90 km of the city of Konya, located in the Central Anatolian region of Turkey. Blood samples were analysed from 46 adult (19 males and 27 females) Holstein cattle suffering from severe theileriosis that were in the progressive stage of the disease. Ages of all animals used in this study ranged from 1.5 to 3 years. A total of 46 clinically healthy Holstein adult (25 males and 21 females) cattle from tick-free farms were used as a control group. They were free of any external, blood, and internal parasites.

Clinical and Parasitological Monitoring of Cattle

The conjunctival, nasal, and oral mucous membranes, prescapular lymph nodes, and rectal temperature of the cattle deemed to be undergoing *T. annulata* infection were examined and biopsies were taken from enlarged nodes. Clinical and parasitological observations were recorded for all the animals showing the clinical signs of *T. annulata* infection. Blood smears were prepared from the ear tip of animals showing poor general health, cachexia, enlarged nodes, and a rise in body temperature (> 39 °C).

Analysis

The smears were air-dried, fixed with methanol, stained with Giemsa stain, and carefully examined under the oil immersion objective of a microscope to estimate the degree of infection. For estimating parasitaemia, the percentage of piroplasm-infected erythrocytes was calculated in 100 cells. Similarly, smears of lymph node biopsies were stained with Giemsa stain and examined for schizonts.

For haematological analysis, approximately 4-ml blood samples were taken from the jugular vein with a syringe containing EDTA. Haematological parameters were determined immediately. For coagulation parameters, blood samples (9 vol.) were collected in 3.8% sodium citrate (1 vol.). The blood collection tubes were kept on ice for no longer than 2 h after blood withdrawal to avoid denaturation of proteins. Platelet-poor plasma was obtained by centrifugation at 1600 xg for 20 min at 4 °C and was stored at –70 °C until analysed.

Reticulocyte count (RTC) was determined by microscopic examination of blood smears stained with new methylene blue staining solution, using the vital stain technique. Haemoglobin (HB) was measured by electronic count, using an Autocounter 920 (SWELAB). In the laboratory, the micro-capillaries were centrifuged at 13,000 rpm for 5 min and the haematocrit values (HCT) were determined directly with a micro-haematocrit reader. The red blood cell count (RBC), white blood cell count (WBC), and platelet count (PLT) were determined by a haemocytometer using Hayem, Turk, and Rees-Ecker solutions, respectively. Haematometric indices were calculated according to the original Wintrobe formulae (MCV = hematocrit x 10/RBC, MCH = haemoglobin x 10 /RBC, and MCHC = haemoglobin x 100/haematocrit). Selected smears were stained with May-Grünwald and Giemsa solution, and then used for differential leucocyte counts.

For the measurement of activated partial thromboplastin time (APTT), plasma (25 μl) was mixed with 25 μl of APTT EA liquid kit (Dialab, Austria) and pre-incubated for 5 min at 37 °C. After adding 25 ml of $CaCl_2$ (0.020 Mol/l) to the mixture, the coagulation time was measured by a coagulometer (DIACLOT C1, Dialab, Austria). To measure prothrombin time (PT), plasma (25 μl) was pre-incubated for 1 min at 37 °C, and after adding 50 μl of thromboplastin (Dialab, Austria) the coagulation time was measured. Plasma fibrinogen was quantified by determining the clotting time with a coagulometer, using a commercial kit (Bovine thrombin, Dialab, Austria).

Statistical Analysis

The results obtained are expressed as mean ± SD. Student's t-test was used to compare the means of the groups. Statements of statistical significance are based on $P < 0.05$.

Results

In this study, there were no clinical or parasitological findings in any of the control cattle. The rectal temperatures of all the cattle were within normal limits. Neither schizonts nor piroplasms were found in any of the control cattle. Analyses for haematological and coagulation parameters were within the acceptable ranges in control cattle; however, *T. annulata* was detected in 46 Holstein cattle examined clinically and parasitologically. The animals showed a high percentage of parasitaemia (64.85%). The presence of schizont-infected cells was also observed in smears of lymph node biopsies (> 5%). Blood-sucking ticks were found on many parts of the cattle and were identified as *Hyalomma* spp. The following clinical observations were recorded: enlargement of the prescapular lymph nodes, pyrexia, inappetence, cachexia, mucous membrane discharge, haemorrhages, dyspnoea, cessation of rumination, protrusion of the eyeball, lacrimation, and conjunctivitis.

In the haematological analyses, statistically significant decreases were observed in the mean red blood cell count, haematocrit value, haemoglobin amount, mean corpuscular haemoglobin concentration, and white blood cell, lymphocyte, neutrophil, monocyte, eosinophil, and basophil counts ($P < 0.05$). Statistically significant increases were seen in the mean corpuscular volume value and reticulocyte count ($P < 0.05$) of infected animals. In addition, activated partial thromboplastin time and prothrombin time were significantly prolonged ($P < 0.05$), and platelet count was significantly lower ($P < 0.05$) in the infected group compared to those in the control group. Table shows the changes in haematological parameters, body temperature, and parasitaemia in the *T. annulata*-infected and control cattle.

Table. The effect of tropical theileriosis on temperature, parasitaemia percentage, and selected haematological and coagulation parameters in Holstein cattle (Mean ± SD).

Parameters	Unit	Control cattle (n = 46)	Infected cattle (n = 46)
APTT	s	64.08 ± 12.63a	71.51 ± 12.46b
PT	s	21.25 ± 1.57a	26.35 ± 2.11b
Fibrinogen	mg/dl	367.46 ± 86.33a	406.26 ± 106.52a
Platelet	$10^3/mm^3$	416.46 ± 113.27a	194.22 ± 79.55b
RBC	$10^6/mm^3$	6.36 ± 1.07a	3.29 ± 0.5b
RTC	$10^6/mm^3$	0.00 ± 0.00a	0.19 ± 0.09b
Haematocrit	%	30.63 ± 4.07a	19.35 ± 2.56b
Haemoglobin	g/dl	9.75 ± 1.64a	5.05 ± 0.94b
MCV	μ^3	48.88 ± 6.74a	59.22 ± 7.50b
MCH	Pg	15.61 ± 3.18a	15.57 ± 3.11a
MCHC	%	32.16 ± 5.85a	26.29 ± 4.80b
WBC	$10^3/mm^3$	8.71 ± 1.41a	4.72 ± 0.82b
Lymphocyte	$10^3/mm^3$	5.10 ± 0.91a	2.79 ± 0.60b
Neutrophil	$10^3/mm^3$	2.85 ± 0.84a	1.46 ± 0.45b
Monocyte	$10^3/mm^3$	0.42 ± 0.11a	0.31 ± 0.08b
Eosinophil	$10^3/mm^3$	0.31 ± 0.07a	0.14 ± 0.05b
Basophil	$10^3/mm^3$	0.03 ± 0.01a	0.01 ± 0.01b
Temperature	ºC	37.6 ± 1.17a	40.49 ± 1.39b
Parasitaemia	%	0.00 ± 0.00a	64.85 ± 11.50b

a, b; different letter in the same row indicates that the value is statistically different from the other ($P < 0.05$).

Discussion

Tropical theileriosis is an important clinical illness of cattle in tropical and sub-tropical countries (2,3). The disease causes significant economic losses as well as reduced production (4,6). *T. annulata* was previously reported from various provinces of Turkey (1,3). In the spring, soon after the appearance of tick infestation, tropical theileriosis begins to appear and reaches its highest level in July (1).

Animals showing a high percentage of parasitaemia and exhibiting clinical signs of tropical theileriosis were included in the study. *T. annulata* spreads through the lymphoid system and other organs rapidly, and induces the production of TNF-α and IFN-α. These cytokines disrupt the physiological integrity of the host. Moreover, the presence of parasites in the pituitary and adrenal glands can cause disturbance of the immune and endocrine systems. It was reported by Forsyth et al. (7) and Glass et al. (8) that the cytokines (TNF-α, IL-1, and IL-6) produced by infected mononuclear cells are responsible for the diverse clinical symptoms of tropical theileriosis, such as depression, pyrexia, anorexia, cachexia, and disseminated haemorrhages. Additionally, symptoms observed in theileriosis are similar to those induced by recombinant bovine TNF-α (9) and high IL-1 (10).

In this study, cattle erythrocytes infected with *T. annulata* had abnormal erythrocyte morphology (Figure 1). Schizonts were found in the lymphocytes of smears of lymph node biopsies (Figure 2). The infected erythrocytes showed cremations, spherocyte formations, and irregular thorn-like protrusions. In theileriosis, these variations in erythrocyte shape are mainly due to parasites in the erythrocytes, erythrocyte oxidation, intravascular thrombi, and immune-mediated processes (11-13).

Reticulocyte count is an indicator of the rate of erythrocyte production. Reticulocytosis is caused by increased RBC destruction. In theileriosis, the reason for this imbalance is that anaemia causes a greater stimulus for RBC production. Singh et al. (12) showed high levels of reticulocyte count in crossbred calves experimentally infected with *T. annulata*-infection; however, Omer et al. (2) did not find reticulocytosis in purebred cattle naturally infected with *T. annulata*. This may be ascribed to differences in stage of disease. The marked increase in the reticulocyte counts in the infected animals supports the contention that reticulocytosis is seen in the later stages of the disease (12).

In the present investigation, red blood cell count, haematocrit value, and haemoglobin concentrations were significantly lower in infected animals compared to control animals. In addition, MCV increased and MCHC decreased (macrocytic hypochromic anaemia) in the infected group compared to those in the control group. While these observations were compatible with those reported by Omer et al. (2) and Beniwal et al. (14), they were in contrast with those reported by Sandhu et al. (15), who showed normocytic normochromic anaemia in calves experimentally infected with theileria. Anaemia occurs in the later stages of theileriosis following
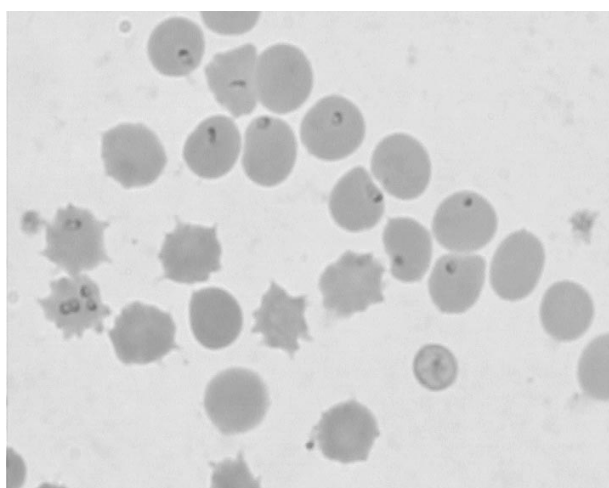

Figure 1. Erythrocyte and piroplasm of *T. annulata* in stained blood smear of cattle.
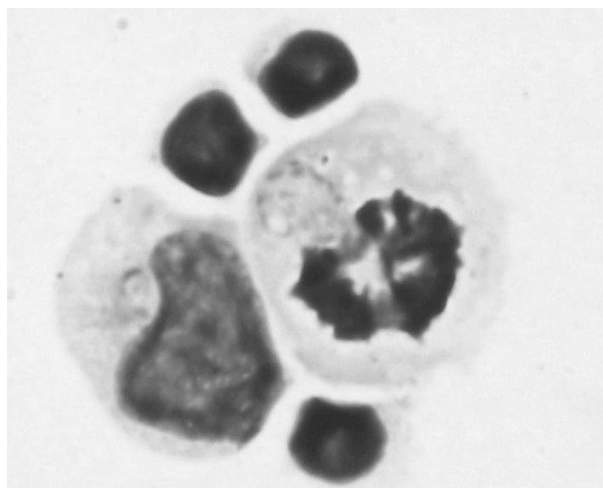

Figure 2. Schizont found in lymphocytes of lymph node biopsy smear.

parasitaemia (12,16-18). Erythrophagocytosis due to an immune-mediated mechanism might be responsible for the erythrocyte destruction (19). Removal of piroplasm-infected erythrocytes by macrophages in the organs of the reticuloendothelial system has been suggested as a cause of anaemia (4,12). In addition, pro-inflammatory cytokines, particularly TNF-α, have been implicated in mediating anaemia associated with tropical theileriosis (7,10). According to Stockham et al. (11) and Omer et al. (2), the decrease in RBC could be due to increased levels of activated complement products. Additionally, since oxidised erythrocytes may be destroyed easily by erythropagocytosis, oxygen radicals may also be involved in the pathogenesis of the anaemia (16,20,21).

Some researchers demonstrated that leucocyte count increased immediately following the theileria infection and then significantly decreased within several days (15,22,23). *T. annulata*-induced leucopoenia is mainly mediated by TNF-α (7). This decrease is related to the destruction of lymphocytes in lymphoid organs and infiltration of these cells into various organs (2,15). In this study, significant decreases were seen in absolute lymphocyte, eosinophil, and neutrophil counts in *T. annulata*-infected cattle compared to those in the control cattle. These findings were consistent with those reported by Omer et al. (2), but although the same researcher reported that there was no significant difference in absolute basophil and monocyte counts between healthy and infected cattle, we detected a marked decrease in both basophil and monocyte counts in the infected group. This variation could be due to differences in stage and severity of disease.

The occurrence of parasites produces lesions in the endothelial lining of blood vessels, tissue damage in organs, such as the liver, kidney, and lung, and multiple petechial haemorrhages (7,24), all of which play an important role in the development of coagulation defects (25). Theileriosis causes hepatic tissue damage, which includes coagulative necrosis, distortion of hepatic cords,

and mononuclear cell infiltration (12,15). In the present study, the alterations in APTT and PT were attributed to impaired hepatic synthesis and the consumption of several pro-coagulant clotting factors in theileriosis. These results are similar to those reported by Singh et al. (12) and Maxie et al. (26), who observed coagulation abnormalities in theileria-infected animals. However, Singh et al. (12) did not find significant prolongation in APTT. The thrombocytopenia in theileriosis was likely to have been caused by increased destruction, consumption and degranulation of platelets in the peripheral blood, and was due to suppressing of the release of platelets from the bone marrow into the blood stream by the parasite and its products (20,27). There was no statistically significant difference in the fibrinogen levels of the 2 groups. Plasma fibrinogen level was found to be slightly higher in the infected group than in the control group in the present investigation. Increased fibrinogen might have been due to an acute-phase reaction that was modulated by the inflammatory response to infections. Similar findings were observed by Stockham et al. (11), who showed high levels of fibrinogen in cows infected with *Theileria buffeli*. During *Theileria annulata* infection, pro-inflammatory cytokines, including TNF-α, IL-1, and IL-6, are released systemically from schizont-infected macrophages and monocytes (7,8). It is possible that cytokines in theileriosis are important mediators in the disturbance of coagulation by stimulating the generation of pro-coagulant activity and by releasing the various inflammatory mediators, such as neutrophil elastase and oxygen free radicals.

Based on the present observations, it can be concluded that severe *T. annulata* infection is associated with profound changes in haematological profiles. The data obtained in this study might form the basis for subsequent studies under natural and experimental field conditions, and it can be considered a useful tool for diagnosis, prognosis, and evaluation of treatment.

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