Bovine Theileriosis: Effects on the Status of Thyroid Hormones, Homocystein, Serum Lipids and Lipoproteins

S.M. Razavi, B. Moghaddas, E. Rakhshandehroo and S. Nazifi
1Department of Pathobiology, 2Department of Clinical Studies, School of Veterinary Medicine, Shiraz University, Shiraz, 71345-1731, Iran

Corresponding Author: S. Nazifi, Department of Clinical Studies, School of Veterinary Medicine, Shiraz University, Shiraz, P.O. Box 1731-71345, Iran Tel: +98-711-2286940 Fax: +98-711-2286950

ABSTRACT

Tropical theileriosis of cattle is a common tick-borne disease in tropical and subtropical regions and is recognized as a disease of major economic importance. This study aimed to determine whether Theileria annulata infection influences the plasma homocystein (Hcy), thyroid activity and serum lipid status in infected cattle. Fifty crossbred Holstein cattle, naturally infected with Theileria annulata were selected and divided into 3 subgroups according to their parasitemia rates (<2, 2-4, 4-8%). Also, 10 non-infected cattle were assigned as controls. Blood samples were collected and hematological parameters, plasma Homocystein (Hcy), thyroid hormones (T₃, T₄, free T₃ (fT₃) and free T₄ (fT₄)) and the concentrations of serum lipid and lipoproteins (cholesterol, triglyceride and lipoproteins including HDL, LDL and VLDL cholesterol) and phosphatidylserine (PS) on erythrocytes were measured. According to the results, significant elevations were observed in the concentration of plasma Hcy (hyperhomocysteinemia) in the infected groups compared to controls. In addition, we conclude that T. annulata can interfere with the lipid metabolism, which is particularly characterized by substantial decreases in the levels of cholesterol, LDL and HDL in the serum of affected animals. In spite of remarkable influences of the parasite on Hcy and lipid contents of the serum, our study proved that tropical theileriosis did not affect the thyroid hormones during parasitemia. This study demonstrates that the infection of cattle with Theileria annulata is mainly characterized by the anemia. Also, evidenced elevation in the level of homocystein (hyperhomocysteinemia) in parasitized cattle can result in oxidative stress on erythrocytes and the probable endothelial injuries. In addition, T. annulata can induce failure in lipid metabolism which is particularly featured by a decrease in the cholesterol, LDL-cholesterol and HDL-cholesterol concentrations; however the parasite cannot implement significant influences on the thyroid hormones in the affected cattle.

Key words: Theileria annulata, homocystein, thyroid hormones, lipids and lipoproteins, cattle

INTRODUCTION

Tropical theileriosis of cattle is a tick-transmitted disease resulting from the infection with the intracellular protozoan parasite Theileria annulata (Ahmed and Mehlhorn, 1999). The infection is widespread, particularly in the Mediterranean Europe, Middle East, India, middle Asia and even China (D'Oliveira et al., 1995), which imposes heavy economic losses (Forsyth et al., 1997).
Homocystein (Hcy) is a highly reactive thiol-containing amino acid produced by the intracellular demethylation of methionine in the methylation process. Every deficiency in vitamins B12, B6 and folic acid supplies or enzymes activity triggers the onset of erythrocyte damage leading to megaloblastic or Biermer anemia (Cristiana et al., 2012). The alterations in the concentration of plasma Hcy, particularly at elevated levels has been also known to produce endothelial cell injury in experimental animals (Han et al., 2015) and also contributes to cardiovascular diseases (CVD) in human (Baggott and Tamura, 2015). Furthermore, it has been reported that homocystein thiolactone changes LDL lipoproteins by binding to apolipoprotein B free lysine groups which could result in elevated LDL aggregations (Naruszewicz et al., 1994). In tropical theileriosis of cattle, although it is clear that the infection with *Theileria annulata* and the invasion of the parasites to the erythrocytes would lead to the occurrence of progressive anemia (Jain, 1993), the probable role of hyper and/or hypo-homocysteinemia in the formation of anemia, as well as its correlations with the serum levels of lipids and lipoproteins remain unclear.

In cattle with tropical theileriosis, some reports stated that schizont-infected cells may disseminate through the lymphoid tissues into pituitary and thyroid glands and cause injury (Forsyth et al., 1999). Moreover, the thyroid metabolism may deteriorate as a result of the decrease in oxygen transport due to anemia (Issi et al., 2011). These conditions affect the concentration of thyroid hormones. On the other hand, variations observed in triglyceride levels, Very Low Density Lipoprotein (VLDL) metabolism, Low Density Lipoprotein (LDL) catabolism and cholesterol synthesis during thyroid affections in human have been attributed to the action of thyroid hormones (Mansourian, 2013).

Phosphatidylserine (PS) is a phospholipid component which predominantly located on the inner membrane leaflet of normal RBC (Vance, 2008). It has been shown that changes in endogenous membrane organization like certain pathologic (Vance, 2008) and apoptotic cells (Utsugi et al., 1991) could lead to express PS on the outer surface.

Despite preceding investigations indicating major changes in the concentration and composition of plasma lipids and lipoproteins in several types of acute conditions (Carpentier and Scruel, 2002) and also phosphatidylserine level (Wanderley et al., 2012), little is known about the pattern of the alterations in the serum lipid components and their relationships with thyroid hormones during bovine tropical theileriosis.

This study was designed to evaluate the levels of plasma homocysteine and serum thyroid hormones and triglyceride, total cholesterol, VLDL-cholesterol, LDL-cholesterol, HDL-cholesterol and phosphatidylserine in different parasitemia rates in cattle naturally infected with *Theileria annulata*.

**MATERIALS AND METHODS**

**Animals and samples:** This study was conducted in the southwest region of Iran (Fars province) with relatively tropical climate condition. In this area, theileriosis due to *Theileria annulata* is prevalent. Two distinct groups were established. The diseased group comprised 50 dairy Holstein cattle, 2-3 years old, naturally infected with *Theileria annulata*, divided into 3 subgroups according to different parasitemia rates (<2, 2-4, 4-8%). Also, ten non-infected cattle served as controls. The infected animals were selected among herds with relatively good nutrition status and body condition scores that kept in free stall barns. However, herds had not a good care for pest management. The animals had not been treated for disease prior to sampling and were screened for other potential causes of anemia by determination of hematological parameters, clinical signs and routine microbiological tests.
Hematological and parasitological measurements: Blood samples were collected from jugular vein into EDTA containing tubes for measuring hematological parameters and homocystein and into plain tubes without anticoagulant for conducting serum assays. Thin blood smears were prepared, fixed with absolute methanol (5 min), stained with 10% Giemsa solution (30 min) and examined under oil immersion (×1000) to observe intraerythrocytic forms of *Theileria annulata*. Piromplasm parasitemia (parasited RBC rate) was also quantified by examination of at least 1×10^4 RBC at a magnification of ×1000 for each case and expressed as the percentage of parasitemia. Identity of *Theileria annulata* was determined on the basis of morphological, clinical and previous epidemiological studies. Hematological parameters were measured by routine standard procedures (Jain, 1993).

Homocystein and thyroid hormones: The blood samples were centrifuged at 1200 g for 10 min at 37°C and the plasma obtained. The enzyme immunoassay (EIA) for the measurement of plasma total homocysteine was performed using the AXIS Homocysteine EIA Kit (Axis-Shield Diagnostic Ltd. Dundee, UK). Triiodothyronine (T3), thyroxine (T4), free T3 (fT3) and free T4 (fT4) levels were measured in the sera specimens by radioimmunoassay kits T3 [^125I], T4 [^125I], fT3 [^125I], fT4 [^125I] (Izotop Co. Budapest, Hungary).

Lipid components analysis: The blood samples were centrifuged at 750 g for 15 min and the sera were separated and kept at -20°C until analysis. The samples with hemolysis were discarded. The analysis of the serum for total cholesterol was done using a commercial kit (Ziest Chem Diagnostics, Tehran, Iran) by a modified Abell-Kendall/Levey-Brodie (A-K) method (Burtis and Ashwood, 1994) and the measurement of serum triglyceride was accomplished based on the enzymatic procedure described by McGowan *et al.* (1983) by the same kit.

Lipoproteins including HDL-cholesterol (mmol L^-1) and LDL-cholesterol (mmol L^-1) were analyzed by quantitative enzymatic colorimetric method using test kits supplied by STANBIO Laboratories, Boerne, TX, USA. All reactions were measured using Digital VIS/Ultraviolet Spectrophotometer (CE 292, series 2, Cecil Instruments, Cambridge England). Also, VLDL-cholesterol was estimated as one fifth of the triglycerides concentration (Friedewald *et al.*, 1972).

The serum concentration of phosphatidylserine was measured by an ELISA Kit for bovine phosphatidylserine (Uscn Life Science Inc., Wuhan). In this method, bovine Annexin A5 is used as a probe in the Annexin A5 affinity assay to detect cells that have expressed phosphatidylserine on the cell surface in the serum by a sandwich enzyme immunoassay. The intensity of the color developed at 450 nm was measured and the serum phosphatidylserine concentration was expressed as nanogram per millilitre.

Statistical analysis: Student’s t-test was used for comparison of measured parameters between control and diseased group. Analysis of variance (ANOVA) and Tukey tests were used for statistical differences between subgroups and Pearson’s correlation coefficients to determine relationships among parameters at different parasitemia rates. Analyses were performed using SPSS software (SPSS Inc., Chicago, USA) version 11.5. All values in the tables were expressed as mean and Standard Error of Mean (SEM) and p<0.05 was considered as statistically significant.
RESULTS

Hematological parameters: The values of hematological parameters in non-infected cattle and those naturally infected with *T. annulata* with different parasitemia rates are shown in Table 1. According to the presented values, our data depict remarkable declines in Red Blood Cells (RBCs), hemoglobin (Hb) concentration and Packed Cell Volume (PCV) in infected cattle rather than controls (p<0.01). This strictly confirms the occurrence of anemia in infected group. In addition, correlation analysis revealed that with the increase in the level of parasitemia, marked decreases were observed in RBC count (r = -0.91, p<0.01), Hb concentration (r = -0.94, p<0.01) and PCV values (r = -0.94, p<0.01), which means higher parasitemia levels coincided with the higher degrees of anemia. On the contrary, no substantial change was found in WBC count between the control and infected groups.

Homocystein and thyroid hormones: The variations occurred in the concentrations of homocystein and thyroid hormones in the infected and healthy cattle are presented in Table 2. In our study, a significant increase was evidenced in the level of homocystein in all infected groups compared to that of the controls. Also, with an increase in the rate of parasitemia, the level of homocystein was elevated in the diseased cattle (r = 0.34, p<0.05). In addition, significant positive correlations were found between the values of homocystein and serum fT₃ (r = 0.30, p<0.05), fT₄ (r = 0.29, p<0.05) and a marked negative correlation with cholesterol (r = -0.32, p<0.05) in infected animals.

According to our data (Table 2), serum thyroid hormones (T₃, T₄, fT₃ and fT4) had no apparent alterations during different levels of parasitemia in infected cattle compared to the controls.

Lipids and lipoproteins: Detailed values of the lipid profile of the serum and also the values related to the expression of phosphatidylserine on RBCs are summarized in Table 3. Accordingly, despite the significant decreases in the mean serum concentrations of the cholesterol and lipoproteins including HDL and LDL cholesterol, the values of triglyceride and VLDL evidenced

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**Table 1: Mean±SEM of hematological parameters in control cattle and those infected with *Theileria annulata* with different parasitemia rates**

| Treatments and parasitemia (%) | RBC (×10¹² L⁻¹) | PCV (L L⁻¹) | Hb (g L⁻¹) | WBC (×10⁹ L⁻¹) | Neutrophil (×10⁹ L⁻¹) | Lymphocyte (×10⁹ L⁻¹) |
|------------------------------|-----------------|--------------|-----------|----------------|----------------------|----------------------|
| **Control**                  |                 |              |           |                |                      |                      |
| 0 (n = 10)                   | 6.64±0.23a      | 0.293±0.016e | 99.1±2.4a | 6.49±0.38a     | 2.10±0.18a           | 4.08±0.21a           |
| <2 (n = 23)                  | 5.71±0.11b      | 0.242±0.022b | 84.1±1.1b | 5.04±0.62ab    | 1.89±0.21b           | 3.21±0.2b            |
| **Diseased**                 |                 |              |           |                |                      |                      |
| 2-4 (n = 16)                 | 4.65±0.18c      | 0.200±0.023b | 71.4±1.3c | 4.27±0.18ab    | 1.75±0.15b           | 3.28±0.13b           |
| 4-8 (n = 11)                 | 2.93±0.21d      | 0.140±0.021d | 48.1±1.7d | 4.46±0.52ab    | 1.64±0.18b           | 3.41±0.19b           |

In each column, control values are assigned as "a". Different letters indicate the statistical significance (p<0.05) among the measurements of the infected cattle in comparison with the controls. RBC: Red blood cells count, PCV: Packed cell volume (hematocrit), Hb: Hemoglobin and WBC: White blood cells

**Table 2: Mean±SEM of the concentrations of homocystein and thyroid hormones in the diseased and control cattle**

| Treatments and parasitemia (%) | T₃ (nmoL L⁻¹) | T₄ (μg dL⁻¹) | fT₃ (pg mL⁻¹) | fT₄ (ng dL⁻¹) | Homocystin (μmoL L⁻¹) |
|------------------------------|--------------|--------------|---------------|---------------|----------------------|
| **Control**                  |              |              |               |               |                      |
| 0 (n = 10)                   | 0.80±0.05³   | 4.43±0.34³   | 4.33±0.28³    | 1.29±0.04³    | 7.34±0.18³           |
| <2 (n = 23)                  | 1.07±0.09¹b  | 3.84±0.33³   | 4.92±0.33³    | 1.41±0.09¹ab  | 11.79±0.24³          |
| **Diseased**                 |              |              |               |               |                      |
| 2-4 (n = 16)                 | 1.08±0.11¹ab | 4.03±0.52³   | 3.74±0.52¹ab  | 1.61±0.009¹ab  | 12.43±0.17³          |
| 4-8 (n = 11)                 | 0.98±0.08⁴b  | 3.53±0.45¹ab | 3.43±0.45¹b   | 1.55±0.05¹b    | 12.51±0.21³          |

In each column, the letter "b" indicate the statistical significance (p<0.05) among the measurements of the infected cattle in comparison with the controls which assigned as “a”
no significant changes in the infected animals compared to the controls. In addition, no considerable relationship was recorded among the lipid components and the parasitemia rate. The mean serum concentrations of the measured lipids and lipoproteins evidenced a statistically constant level during different parasitemias in the diseased subgroups as well.

This investigation showed that the serum concentration of phosphatidylserine increased in the infected cattle in comparison with control. Also, it was evident that the serum concentration of phosphatidylserine was strongly elevated in proportion to the increasing parasitemia rate in the diseased animals ($r = 0.56$, $p<0.01$).

DISCUSSION

Bovine tropical theileriosis caused by *T. annulata* is a serious haemoprotozoan disease of cattle that occurs in tropical and sub-tropical countries. The results of our study investigating the blood parameters of cattle infected with different rates of parasitemia clearly demonstrated that the infection of cattle with *Theileria annulata* can induce impairments in the lipid metabolism and enhance the production of homocystein (hyperhomocysteinemia), a condition that has been proven to be involved in cardiovascular diseases (CVD) (Baggott and Tamura, 2015) or endothelial cell damage (Han *et al*., 2015).

Significant decreases in hematological parameters including RBC count, PCV and hemoglobin (anemia) were confirmed in the infected cattle. Although this finding has been discussed in the cases of bovine (Shiono *et al*., 2003; Razavi *et al*., 2011) and ovine theileriosis (Nazifi *et al*., 2011), the underlying mechanisms of such a progressive anemia are still not clearly understood. However, low levels of RBCs, PCV and hemoglobin concentration in bovine theileriosis due to *T. annulata* have been attributed to erythrocytes destruction by macrophages in the lymph nodes, spleen and other organs of the monocyte-macrophage system (Singh *et al*., 2001) and also one recent hypothesis indicates the interference of the parasite with protective antioxidant mechanisms of RBCs against oxidative damages (Shiono *et al*., 2003; Razavi *et al*., 2011).

These findings revealed significant elevations in the concentration of plasma homocystein (hyperhomocysteinemia) during parasitemia in *T. annulata* infected cattle. Although there have been no documented or incisive investigations on homocystein changes in blood parasites of animals, several publications on human cardiovascular diseases (CAD) correlate hyperhomocysteinemia with coronary, cerebral and peripheral artery disease, as well as venous thrombosis (Nygard *et al*., 1997; Ganguly and Alam, 2015). In addition, the pathogenesis of the vascular injury caused by an increase of Hcy includes damage to the endothelial cell, increased oxidation of LDL-cholesterol with deposits in the vessel wall and direct activation of the coagulation cascade (Durand *et al*., 2001). On the other hand, Hcy has been demonstrated to increase oxidative
stress through autoxidation of Hcy yielding hydrogen peroxide (Loscalzo, 1996). Thus, the elevation of Hcy in parasitized cattle in our study can also corroborate the results of preceding studies that implicate the role of oxidative stress on damaging erythrocytes and the anemia (Nazifi et al., 2011; Razavi et al., 2011), as well as emphasize the probable formation of endothelial injuries and coagulation disorders (like disseminated intravascular coagulation) due to occurred hyperhomocysteinemia, which, in turn, could help the appearance of anemia.

In the present study, thyroid hormones did not considerable changes in the diseased cattle. According to previous studies, schizont-infected cells may disseminate through the lymphoid tissues into pituitary and thyroid glands and cause injury and reduce their secretions (Forsyth et al., 1999). It has been reported that following experimental infection with T. annulata, thyroid hormones decreased significantly (Garg et al., 2001; Khalil et al., 2011). Reduced thyroid secretion rate during feed deprivation has also been reported in farm animals (Garg et al., 2001) and it has been postulated that the lower level of T₃ and T₄ could partly be due to the anorexia condition prevailing in the disease. In addition, several trace elements are needed for the normal function, synthesis and metabolism of thyroid hormones. In particular, it has been indicated that deficiencies in the levels of selenium (Beckett and Arthur, 2005) and zinc (Baltaci et al., 2004) have a suppressing effect on thyroid hormones. Thus, it seems that having a good nutrition could be a reason for unchanged levels of thyroid hormones in the infected animals.

The substantial decreases in the levels of cholesterol, LDL and HDL content of the serum in the infected cattle apparently proved that the infection with T. annulata can induce impairments in lipid metabolism. However, in view of the fact that no remarkable correlations were found between the parasitemia rate and the levels of serum lipids and lipoproteins and also statistically constant levels of those factors in the sera of the diseased cattle, it can be stated that the lipid components of the serum are not directly influenced by the parasites during parasitemia. In other words, some other determinative factors (probably the compensatory role of the liver) arise to balance the lipid profile of the serum during bovine tropical theileriosis; however, these circumstances require further studies.

Reducions in the lipid component of the serum in anemia caused by haemoparasites have been reported by Elissalde et al. (1983). Also, Singh et al. (2001) and Yagi et al. (1992) have shown significant decreases in the concentration of cholesterol and triglycerides in bovine theileriosis. They affirmed that the observed decreases could be assigned to the anorexia, with the high rise of temperature and diarrhea causing impaired absorption of fatty acids. According to preceding studies, our results were not in line with Omer et al. (2003) who did not show any significant changes in cholesterol levels in cattle suffering from tropical theileriosis. Moreover, Yadav and Sharma (1986) recorded a marked elevation in cholesterol level in experimentally T. annulata infected cattle and stated this increase was probably due to the liver damage. These controversial results may be attributed to the different (low or high level) parasitemia rates in cattle whose serum lipid profiles had been studied or the interval time during the onset of anemia to blood sampling.

In this study, the serum concentration of phosphatidylserine (PS), as a marker of membrane damage, in infected animals and also the strong positive correlation between the serum concentration of PS and the level of the parasitemia in cattle infected with T. annulata could support the previous hypothesis that the oxidative injuries of erythrocytes could induce the development of the anemia in tropical theileriosis.
Taken together, this study demonstrates that the infection of cattle with *Theileria annulata* is mainly characterized by the anemia. Also, evidenced elevation in the level of homocysteine (hyperhomocysteinemia) in parasitized cattle can result in oxidative stress on erythrocytes and the probable endothelial injuries. In addition, *T. annulata* can induce failure in lipid metabolism which is particularly featured by a decrease in the cholesterol, LDL-cholesterol and HDL-cholesterol concentrations; however the parasite cannot implement significant influences on the thyroid hormones in the affected cattle.

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