Bull Vet Inst Pulawy 51, 15-18, 2007

CHANGES IN SELECTED SERUM COMPONENTS IN CATTLE NATURALLY INFECTED WITH *THEILERIA ANNULATA*

RAMAZAN COL, AND UGUR USLU[1]

Department of Physiology, [1]Department of Parasitology, Faculty of Veterinary Medicine,
University of Selcuk, 42031, Campus, Konya, Turkey
rcol@selcuk.edu.tr

Received for publication December 21, 2006.

Abstract

Changes in selected serum components were investigated to elucidate metabolic profile in cattle naturally infected with *T. annulata.* Statistically significant increases were observed in the mean serum activity of aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transferase, bilirubin, creatinine, urea, and creatinine kinase, and statistically significant decreases were seen in the mean serum contents of glucose, total protein, albumin, triglycerides, cholesterol, calcium, and phosphorus, along with a non-significant decrease in iron level in infected animals when compared with controls (uninfected). The study has shown that *T. annulata* infection in cattle is associated with profound biochemical changes.

Key words: cattle, *Theileria annulata*, serum biochemistry.

Tropical theileriosis, is caused by the protozoan parasite *Theileria annulata*, and transmitted by *Hyalomma* sp., is a severe disease of cattle in endemic areas from the Mediterranean basin to China, which causes serious economic losses through mortality and loss of productivity (1, 6). *T. annulata* sporozoites infects the host mononuclear cells (macrophages/monocytes and B lymphocytes) in the lymph nodes draining the site of inoculation of the sporozoites by ticks. The sporozoites transform into schizonts in the mononuclear cells. Host cells become transformed and proliferate in synchrony with the parasite during this process, named the macroschizont stage (7). The schizonts undergo further differentiation to merozoites, which are released by the lysis of the infected cells. Later, the merozoites invade red blood cells. It is followed by the development of piroplasms in erythrocytes, and these intra-erythrocytic piroplasms become available to the vector (3, 5).

Plasma biochemistry may be an indication of the severity of the infection, and a very good tool for diagnosis, prognosis, and evaluation of the therapy applied. Also, to understand the host-parasite relationship at a molecular level and to describe the disease clearly, some biochemical parameters may be determined. Based on the above considerations, the present study was designed to investigate the influence of the tropical theileriosis on some serum components associated with lipid, protein, and carbohydrate metabolism in Holstein-Friesian cattle naturally infected with *T. annulata*, and to relate the changes to the severity of the infection.

Material and Methods

The present investigation was carried out during the disease seasons from May 2004 to July 2005, in 4 villages around the Konya Country, located in the Central Anatolian region of Turkey. These villages (Central Konya, Kadınhanı, Sarayönü and Çumra) were within 10-90 km of Konya, in different directions from the city. The serum samples from 46 adult (19 males and 27 females) Holstein-Friesian cattle with sever theileriosis, in the late stage of the disease, were analysed. The age for all of the animals used in this study ranged from 1.5 to 3 years. A total of 46 clinically healthy, Friesian-Holstein adult (25 males and 21 females) cattle from tick-free farms, were used as a control group.

Clinical and parasitological observations were recorded from all the animals showing symptoms of acute clinical theileriosis. The conjunctiva, nasal, and oral mucous membranes were examined. Animals showing a rectal temperature above 39ºC were considered to be suffering from fever. Biopsies were taken from enlarged nodes. Schizonts in lymph node biopsy smears and piroplasms in blood smears were determined. Thin blood smears were prepared from sick cattle, fixed with methanol, and stained with Giemsa dye. For estimating parasitaemia, the percentage of piroplasm-infected erythrocytes was calculated in 100 cells. Similarly, lymph node biopsy smears were stained with Giemsa stain and examined for schizonts. The results were recorded in percentages.

For biochemical analyses, samples of 5 ml of blood were allowed to clot and the serum, after centrifugation, was stored in single test tubes at –18°C until processing. Serum concentrations of cholesterol, triglycerides, bilirubin, creatinine, creatinine kinase (CK), glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ- glutamyl transferase (GGT), urea, total protein (TP), albumin (Alb), iron (Fe), calcium (Ca), and phosphorus (P) were determined by a computer process-controlled multiparametric autoanalyser (Tokyo Boeki, TMS 1024) using commercial kits (Sprinreact SA, Spain).

Statistical analysis. The results obtained were expressed as mean ±SD. Student's *t*-test was used to compare the mean data between groups. Statements of statistical significance were based on $P<0.05$ (13).

Results

In the present investigation, the following findings for the clinical condition of the cattle were assessed: gross enlargement of the right prescapular lymph nodes, temperature >39.5°C, inappetence, cachexia, mucous membrane discharge, haemorrhages, recumbent, dyspnoea, cessation of rumination, protrusion of the eyeball, lacrimation, and conjunctivitis. Blood-sucking ticks were found on many body parts of the cattle and were identified as *Hyalomma* sp. The animals showed a high percentage of parasitaemia (64.85%). The presence of schizont-infected cells was also observed in lymph node biopsy smears (>5%).

In the analysis of serum of the infected animals, when compared with controls, it was found that there were statistically significant increases ($P<0.05$) in the mean serum activity of aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transferase, and creatinine kinase, and in serum content of bilirubin, creatinine, and urea. On the other hand, statistically significant decreases ($P<0.05$) were seen in the mean serum contents of glucose, total protein, albumin, triglycerides, cholesterol, calcium, and phosphorus, along with a non-significant decrease in iron level ($P>0.05$). Additionally, changes in serum biochemical parameters in the infected animals and control group are given in Table 1.

Discussion

Bovine tropical theileriosis caused by *T. annulata* is a serious haemoprotozoan disease of cattle in tropical and sub-tropical countries. The disease is seen as a widespread one in Turkey (1, 9, 18). In spring in Turkey, tropical theileriosis begins to appear soon after the appearance of tick infestation, and reaches the highest prevalence in July (11).

T. annulata spreads through the lymphoid system and other organs rapidly and induces the production of TNF-α and IFN-a. These cytokines disrupt the physiological integrity of the host. Additionally, 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.* (5) and Glass *et al.* (6) that the cytokines (TNF-α, IL-1 and IL-6) produced by infected mononuclear cells are responsible for the diverse clinical signs of theileriosis such as depression, pyrexia, anorexia, cachexia, and disseminated haemorrhages. Moreover, symptoms observed in theileriosis are similar to those induced by recombinant bovine TNF-α (2) and high IL-1 (7).

Table 1
Effect of tropical theileriosis on mean selected serum biochemical parameters in Holstein-Friesian cattle

Parameter	Unit	Control cattle (n=46)	Infected cattle (n=46)
AST	U/L	67.46±19.86 a	124.61± 23.03 b
ALT	U/L	34.83±11,65 a	69.87±12.78 b
GGT	U/L	24.41±9.76 a	40.57±18.08 b
CK	U/L	141.37± 44.31 a	589.57± 243.72 b
Creatinine	mg/dL	0.97±0.29 a	1.45±0.41 b
Urea	mg/dL	18.70±8.32 a	27.24±8.74 b
bilirubin	mg/dL	0.17±0.10 a	0.66±0.24 b
TP	g/dL	7.87±0.86 a	6.02±0.98 b
Alb	g/dL	3.76±0.68 a	2.97±0.60 b
Triglycerides	mg/dL	17.43±8.42 a	11.69±7.59 b
Cholesterol	mg/dL	150.15± 34.06 a	104.70± 36.78 b
Glucose	mg/dL	57.46±11.86 a	36.15±8.97 b
Fe	μg/dL	108.07± 34.88	94.07±33.23
Ca	mg/dL	9.82±1.59 a	7.65±1.93 b
P	mg/dL	5.28±0.95 a	4.17±1.04 b

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

The occurrence of parasites in any tissue causes the parasitic tissue damage. In hepatic injury seen in theileriosis, increased serum activities of AST and ALT are closely associated with the hepatic function (5). Additionally, GGT is an enzyme commonly used as a sensitive indicator of liver disease and it increases in *T. annulata*-infected animals (10). Furthermore, the significant rise in serum AST and CK activities is due to muscle trauma caused by prolonged recumbency in theileriosis.

In the present study, significant increase in biliribun concentration in infected animals compared to controls was observed. According to Sandhu *et al.* (10), rise in bilirubin levels is due to the destruction of parasitized erythrocytes by erythrophagocytosis in the spleen, lymph nodes, and other organs of the reticulo-endothelial system. In addition, Omer *et al.* (8) attributed the increase in bilirubin to hepatic dysfunction and haemolytic anaemia. Moreover, it was observed in this study that infected cattle had higher concentrations of serum creatinine. While our observation contradicts that of Omer *et al.* (8), who showed significant decrease in cattle naturally infected with *T. annulata*, it was consistent with data reported by Yeruham *et al.* (17), who attributed the increase in creatinine to damage observed in the liver and kidneys in babesisosis in sheep. The increase in urea level was similar to that reported by Singh *et al.* (12) and Sandhu *et al.* (10). However, Omer *et al.* (8) showed a non-significant increase in cattle naturally infected with *T .annulata*. A rise in urea levels might be attributed to kidney damage.

In this study, infection caused an apparent decrease in the concentration of serum glucose. This observation is in agreement with that of Yadav and Sharma (16) who reported that hypoglycaemia could be due to the utilisation of glucose by parasites and damage to the liver in cattle infected with *T. annulata*, but contradicts to that of Sandhu *et al.* (10), who showed a non-significant decrease in calves infected with *T. annulata.* Moreover, infected animals in the present study developed hypoalbuminaemia and hypoproteinaemia. This finding supports the results of Yadav and Sharma (16) and Singh *et al.* (12) obtained in their studies on theileriosis. However, Sandhu *et al.* (10) found only non-significant decreases in these parameters. Stockham *et al.* (14) reported that decreases in protein and albumin concentrations could be due to the extravascular accumulation of proteinaceous fluids, resulting from diseased lymph nodes, and thus explained the oedema and body cavity effusions. Furthermore, Singh *et al.* (12) and Omer *et al.* (8) attributed the decrease in serum protein to hypoalbuminaemia and hypoglobulinaemia arising from liver failure. Observation of a decrease in serum cholesterol and triglycerides in *Theileria* and *Babesia* infections in previous studies confirms our present findings (4, 12). These decreases in cholesterol and triglyceride levels may be ascribed to anorexia and diarrhoea. However, Yadav and Sharma (16) recorded a marked increase in cholesterol level in experimentally *T. annulata*-infected cattle from day 0 to day 15, followed by a sudden fall, reaching values below the pre-infection level by day 40. According to Yadav and Sharma (16), this could be due to liver damage that results in a concurrent increase in the level of fats with the reduction of sugar and protein. Additionally, Omer *et al.* (8) did not show significant change in cholesterol levels in infected cattle when compared to the control cattle.

In the present study, significant decreases in serum calcium and phosphorus in infected animals compared to the controls were consistent with data reported by Omer *et al.* (8). In *T. annulata* infection of cattle, hypocalcaemia was probably due to the hypoproteinaemia, decreased dietary intake, intestinal malfunction, and kidney damage. The decreased serum phosphorus concentration in cattle with theileriosis is the result of diarrhoea and renal wasting. In the present investigation, we found that the concentration of iron in *Theileria*-infected cattle showed only non-significant decrease. While Watanabe *et al.* (15) demonstrated remarkable increases in serum iron level in calves infected by *T. sergenti*, Omer *et al.* (8) in *Theileria*-infected cattle reported decreases. Because haemoglobinuria has never been observed in calves infected with *Theileria*, no substantial loss of body iron amount is thought to occur.

In the present investigation, it was concluded that some biochemical values were greatly influenced by *T. annulata* infection in cattle. These changes are important as they may indicate the extent of tissue damage and help in better understanding the pathogenesis of the disease. The data obtained in this study might form an indicative basis for subsequent studies under natural and experimental field conditions, and it should be used as a useful tool for diagnosis, prognosis, and evaluation of the therapy applied.

References

1. Aktas M., Dumanli N., Angin M.: Cattle infestation by *Hyalomma* ticks and prevalence of *Theileria* in *Hyalomma* species in the east of Turkey. Vet Parasitol 2004, **119**, 1–8.

1.2. Bielefeldt Ohmann H., Campos M., Snider M., Rapin N., Beskorwayne T., Popowych Y., Lawman M.J., Rossi A., Babiuk L.A.: Effect of chronic administration of recombinant bovine tumor necrosis factor to cattle. Vet Pathol 1989, **26,** 462–472.

3. Campbell J.D.M., Spooner R.L.: Macrophages behaving badly: infected cells and subversion of immune responses to *Theileria annulata*. Parasitol Today 1999, **15**, 10-16.

4. Elissalde G.S., Wagner G.G., Craig T.M., Elissalde M.H., Rowe L.: Hypocholesterolemia and hypocortisolemia in acute and terminal *Babesia bovis* infection. Vet Parasitol 1983, **12,** 1-11.

5. Forsyth L. M. G., Minns F. C., Kirvar E., Adamson R. E., Hall F. R., McOrist S., Brown C. G. D., Preston P. M.: Tissue damage in cattle infected with *Theileria annulata* accompanied by metastasis of cytokine-producing, schizont-infected mononuclear phagocytes. J Comp Path 1999, **120**, 39–57.

6. Glass E.J., Craigmile S.C., Springbett A., Preston P.M., Kirvar E., Wilkie G.M., Eckersall P.D., Hall R. F., Brown C.G.D.: The protozoan parasite, *Theileria annulata*, induces a distinct acute phase protein response

in cattle that is associated with pathology. Int J Parasitol 2003, **33,** 1409–1418.

7. Graham S.P., Brown D.J., Vatansever Z., Waddington D., Taylor L.H., Nichani A.K., Campbell J.D.M., Adamson R.E., Glass E.J., Spooner R.L.: Proinflammatory cytokine expression by *Theileria annulata* infected cell lines correlates with the pathology they cause *in vivo*. Vaccine 2001, **19,** 2932–2944.

7.8. Omer O.H., El-Malik K.H., Magzoub M., Mahmoud O.M., Haroun E.M., Hawas A., Omar H.M.: Biochemical profiles in Friesian cattle naturally infected with *Theileria annulata* in Saudi Arabia. Vet Res Commun 2003, **27,** 15-25.

9. Omer O.H., El-Malik K.H., Mahmoud O.M., Haroun E.M., Hawas A., Sweeney D., Magzoub M.: Haematological profiles in pure bred cattle naturally infected with *Theileria annulata* in Saudi Arabia. Vet Parasitol 2002, **107,** 161–168.

9.10. Sandhu G.S., Grewal A.S., Singh A., Kondal J.K., Singh J., Brar R.S.: Haematological and biochemical studies on experimental *Theileria annulata* infection in crossbred calves. Vet Res Commun 1998, **22,** 347-354.

9.11. Sayin F., Dinçer S., Karaer Z., Cakmak A., Inci A., Yukari B.A., Eren H., Vatansever Z., Nalbantoglu S.: Studies on the epidemiological of tropical theileriosis (*Theileria annulata* infection) in cattle in Central Anatolia, Turkey. Trop Anim Health Prod 2003, **35,** 521-539.

12. Singh A., Singh J., Grewal A.S., Brar R.S.: Studies on some blood parameters of crossbred calves with experimental *Theileria annulata* infection. Vet Res Commun 2001, **25,** 4, 289-300.

12.13. SPSS: SPSS/PC+V.2.0, Base Manual for IBM PC/XT/AT and PS/2. Marija and Morusis, Chicago, IL, Soil Science Society of America, Inc., 1988.

14. Stockham S. L., Kjemtrup A. M., Conrad P. A., Schmıdt D. A., Scott M. A., Robınson T. W., Tyler J. W., Johnson G. C., Carson C. A., Cuddihee P.: Theileriosis in a Missouri beef herd caused by *Theileria buffeli*: Case report: herd investigation, ultrastructure, phylogenetic analysis, and experimental transmission. Vet Pathol 2000, **37**, 11–21.

15. Watanabe K., Ozawa M., Ochial H., Kamohara H., Ijima N., Negita H., Orino K., Yamamoto S.: Changes in iron and ferritin in anemic calves infected with *Theileria sergenti*. J Vet Med Sci 1998, **60,** 8, 943-947.

15.16. Yadav C.K., Sharma N.N.: Changes in blood chemical components during experimentally induced *Theileria annulata* infections in cattle. Vet Parasitol 1986, **21,** 91-98.

17. Yeruham I., Hadani A., Galker F.: Some epizootical and clinical aspects of ovine babesiosis caused by *Babesia ovis* - a review. Vet Parasitol 1998, **74,** 153-163.

18. Zhang Z.H.: A general review on the prevention and treatment of *Theileria annulata* in China. Vet Parasitol 1997, **70,** 77-81.