*Bulgarian Journal of Agricultural Science, 22 (No 2) 2016, 303–307*
*Agricultural Academy*

# CHANGES IN SOME BIOCHEMICAL PARAMETERS IN SMALL RUMINANT THEILERIOSIS

N. GUNES[*1], D. ARSOY[2], M. AKTAS[3], S. OZUBEK[3], M. OZUICLI[4], L. AYDIN[4] and O. SELCUK[4]
[1] *Uludag University, Faculty of Veterinary Medicine, Department of Biochemistry, Gorukle Campus, 16059, Bursa Turkey*
[2] *Near East University, Faculty of Veterinary Medicine, Department of Zootecnics, 99138, Lefkoşa, Cyprus*
[3] *Fırat University, Faculty of Veterinary Medicine, Department of Parasitology, 23200, Elazığ, Turkey*
[4] *Uludag University, Faculty of Veterinary Medicine, Department of Parasitology, Elazığ Campus, 16059, Bursa, Turkey*

## Abstract

N. GUNES, D. ARSOY, M. AKTAS, S. OZUBEK, M. OZUICLI, L. AYDIN and O. SELCUK, 2016. Changes in some biochemical parameters in small ruminant theileriosis. *Bulg. J. Agric. Sci.*, 22: 303–307

The aim of this study was to determine changes in some biochemical parameters in the healthy and infected sheeps and goats with Theileriosis. Sixty-five sheep and 67 goats were used in the study. Blood samples were tested for the presence of Theileriosis using PCR and RLB. 3.07% of sheep and 3.06% of goats blood samples were found positive with Theileria ovis. We observed decreases in the levels of total protein, albumin, glucose and increases in ALT and AST values in the infected animals compared with the healthy group. This study suggests that changes in some biochemical parameters may occur and in terms of PCR can be detected in infected animals that are in the early stage of disease and do not show any clinical symptoms.

*Key words:* Biochemical parameter, Small ruminant, PCR, Theileriosis

## Introductıon

Theileriosis and babesiosis are protozoal diseases of ruminants that cause big economic losses all around the world. *Theileria lestoquardi*, *T. ovis* and *T. separata* are recognized as the species that cause ovine theileriosis (Uilenberg, 2001; Altay et al., 2008; Nagore et al., 2004; Nambata et al., 1994; Irshad et al., 2010). Generally, the diagnosis of ovine and caprine piroplasmosisi is based on morpholojical examination of blood smears and clinical symptoms. These metods are useful in acute cases, but are insufficent for carrier animals (Almeria et al., 2001; Inci et al., 2010). Polymerase chain reaction (PCR) is the most commonly used molecular technique for detecting piroplasms in recent years, but it fails to detect mixed infections. Another technique known as reverse line blot (RLB) has been developed to detect all the piroplasm species that infect sheep and goat populations. RLB has been proven to be a very valuable tool for identifying undocumented sequences and diversity (Nagore et al., 2004; Schnittger et al., 2004; Georges et al., 2001; Sivakumar et al., 2014; Aktas et al., 2005).

The blood biochemical profile sould be determined in order to diagnose the disease. Blood parameters such as glucose, urea, cholesterol, albumin, globulin, total protein and total lipid concentrations besides some enzymes activities such as AST (Aspartat amino transferase), ALT (Alanin amino transferase), ALP (Alkaline phosphatase) are among important biochemical parameters in determining the biochemical profile (Dede et al., 2014).

The aim of the present study was to determine the molecular prevalence of subclinical piroplasmosis of goats and sheep feeding in the same grazing land of Northern Cyprus by PCR and RLB techniques and to examine the changes of some biochemical parameters in these clinically normal small ruminants. The reason for selecting this region is suitable season for tick populations throughout the year. In addition, these studies have not been done in this region.

**E-mail:* ngunes@uludag.edu.tr

## Materıals and Methods

### *Study Area*

Northern Cyprus has an area of 3355 square miles (8689 km$^2$), which amounts to around a third of the island. 75 kilometres (47 mi) to the north of Northern Cyprus lies Turkey with Syria lying 97 kilometres (60.3 mi) to the east. It lies between latitudes 34° and 36°N, and longitudes 32° and 35°E. The winter in Northern Cyprus is cool and rainy, particularly between December and February, with 60% of annual rainfall. These rains produce winter torrents that fill most of the rivers, which typically dry up as the year progresses Climate conditions on the island vary by geographical factors. The Mesaoria Plain, cut off from the summer breezes and from much of the humidity of the sea, may reach temperature peaks of 40 to 45°C (104 to 113°F). Humidity rises at the Karpaz Peninsula. Humidity and water temperature, 16 to 28°C (61 to 82°F), combine to stabilize coastal weather, which does not experience inland extremes (Maric, 2009).

### *Animal Materials*

Blood samples used in the study were obtained from animals that are raised in three natural, free-range farms that are an average of 50 km from each other in November 2013. These farms are located far from areas in which organized breeding enterprises operate. Based on the location of these farms, the animals used in this study can be considered a representative sample of all Northern Cyprus. Sixty-five sheep and 67 goats that are 2 to 3 years of age were used in the study. Two of these farms raise both sheep and goats, while one of them raises only goats. Sheep are Awassi x Gum crossbred, whereas goats belong to the Damascus breed.

### *DNA Extraction*

Blood samples were defrosted and homogenized at room temperature for 10-15 seconds. The genomic DNA was extracted from 200 µl of EDTA anticoagulated blood with a QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. DNA was eluted in 100 µl elution buffer and stored −80°C until further analysis.

### *Polymerase chain reaction and agorose jel electrophoresis*

For the amplification of *Theileria* species, one set of primers were used to amplify an approximately 360–430 bp fragment of the hypervariable V4 region of the 18S rRNA gene. The forward [RLB-F2 (5'-GACACAGGGAGGTAGTGACAAG-3')] and the reverse [RLB-R2 (Biotin-5'-CTAAGAATTTCACCTCTGACAGT-3')] primers were described by Georges et al (Georges et al., 2001). Touchdown PCR is a modification of conventional PCR.

### *Revers line blotting (RLB)*

Probes contain N-terminal *N*-(trifluoracetamidohexyl-cyanoethyl,*N*,*N*-diisopropyl phosphoramidite [TFA])-C6 amino linker. Biodyne C membrane was activated in 10 ml of 16% EDAC 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide for 10 min at room temperature and placed in a miniblotter after washed demineralised water. The probes included on the membrane are presented in Table 1.

**Table 1**

**Sequence of oligonucleotide probes hybridised on the membrane**

| Probe | Sequence of oligonucleotide (5'-3') | Reference |
|---|---|---|
| Catchall | TAATGGTTAATAGGA(AG)C(AG)GTTG | Gubbels et al.,1999 |
| *Theileria* spp. | TGATGGGAATTTAAACC(CT)CTTCCA | Nagore et al., 2004 |
| *Theileria* sp. OT1 | ATC TTC TTT TTG ATG AGT TGG TGT | Nagore et al., 2004 |
| T. ovis | TTTTGCTCCTTTACGAGTCTTTGC | Nagore et al., 2004 |
| *Theileria* sp. OT3 | ATTTTCTCTTTTTATATGAGTTTT | Nagore et al., 2004 |
| T. lestoquardi | ATTGCTTGTGTCCCTCCG | Schnittger et al., 2004 |
| *Theileria* sp. MK | CATTGTTTCTTCTCATGTC | Altay et al., 2007 |
| *Theileria* sp. China 1 | TCGGATGATACTTGTATTATC | Schnittger et al., 2004 |
| *Theileria* sp. China 2 | TGCATTTTCCGAGTGTTACT | Schnittger et al., 2004 |
| *B. ovis* | GCGCGCGGCCTTTGCGTTACT | Nagore et al., 2004 |
| *B. motasi* | ATTGGAGTATTGCGCTTGCTTTTT | Nagore et al., 2004 |
| *B. crassa* | TTA TGG CCC GTT GGC TTA T | Schnittger et al., 2004 |

*Table Reference*: Altay, K., Dumanli, N., Aktas, M., 2007. Molecular identification, genetic diversity and distribution of *Theileria* and *Babesia* species infecting small ruminants. Vet. Parasitol. 147, 161–165

***Biochemical study***

Blood samples were collected from animals that were subjected to similar living conditions and feeding on natural grazing lands of North Cyprus. Blood were obtained vena jugularis via sterile vacutainer to anticoagulant free and heparinised sterile tubes. Blood samples were transferred to the laboratory under cold storage conditions and sera were seperated by centrifugation. Glucose, Ca and inorganic phosporus levels were immediately analyzed. Sera were kept at -20°C for later analyses of total protein, albumin, total cholesterol, ceruloplasmin levels and AST, ALT activities. Total protein, albumin (Diachem LTD, Hungary), total cholosterol (Biolabo SA, Maizy-France), glucose, calcium, inorganic phosporus (Teco Diagnostic, U.S.A) concentrations and AST and ALT (Cormay, Germany) activities were determined by spectrophotmetry (Schimadzu UV-1601) using commercial kits. Ceruloplasmin level was determined as described by Sunderman et al. (1970).

Statical analyses were performed by SPSS MS Windows Release 22.0 software using t-test.

***Results***

Blood samples were examined for Theileriosis by PCR and RLB. 3.07% of sheep and 3.06% of goats blood samples were found positive with *Theileria ovis*. In addition, blood samples were examined by parasitic diseases in terms of babesiosis, but not observed any Babesia spp. Based on these results samples were divided healthy and infected groups. We observed decreases in the levels of t.protein, albumin, glucose and increases in ALT and AST values in the infected animals compared with the healthy group. The biochemical results are summarized in Table 2.

## Dıscussıon

Blood smears and clinical symptoms are useful in acute cases of piroplasmosis, but reinsufficient in subclinical cases. As opposed to these conventional methods, moleculer methods would allow direct ,specific and sensitive detection of parasites. PCR is the most commonly used molecular technigue, but is not capable of detecting mixed infections. Therefore, a more sensitive molecular technique, known as RLB, was developed to detect species-specific infections and mixed infections (Inci et al., 2010). In this study, 3.07% of sheep and 3.06% of goats blood samples were found positive with Theileria ovis. In addition, blood samples were examined by parasitic diseases in terms of babesiosis, but not observed any Babesia spp.

We found significant changes in albumin and total protein levels between healthy and infected animals that fell outside of the reference values reported for these species. However, changes observed in glucose levels, and ALT and AST activites between the groups remained within the reference values (Table 2). T. cholesterol, Ca and IP, ceruloplazmin levels were not significantly different between groups.

It is well-established that culture breed animals are more susceptible to diseases caused by parasites than native

**Table 2**

**Some serum biochemical values in healthy and infected sheep and goats**

| | Sheep | | Referance Values | Goat | | Referance values |
|---|---|---|---|---|---|---|
| | Healthy X±Sx | Infected X±Sx | | Healthy X±Sx | Infected X±Sx | |
| T. Protein (g/dl) | 6.63±0.33a | 5.30±0.35b | 5.9–7.8 | 6.42±0.30a | 5.25± 0.25b | 6.1–7.5 |
| Albumin (g/dl) | 3.45±0.14a | 2.21± 0.11b | 2.7–3.7 | 3.13± 0.10a | 2.20± 0.12b | 2.3–3.6 |
| Glucose (mg/dl) | 88.23±6.99a | 64.29±6.24b | 44–81 | 58.36±3.92a | 47.64±2.50b | 48–76 |
| Total Cholesterol (mg/dl) | 145.81±5.64 | 152.34±6.28 | 44–90<br>100–150* | 111.98±8.38 | 120.61± 5.12 | 65–136 |
| Ca (mg/dl) | 8.23±0.70 | 8.05±0.75 | 9.3–11.7 | 8.14± 0.85 | 7.98±0.72 | 9.0–11.6 |
| I.P (mg/dl) | 3.66±0.65 | 3.27±0.51 | 4.0–7.3 | 4.14±0.56 | 3.68±0.51 | 3.7–9.7 |
| Ceruloplasmin (mg/dl) | 23.0±2.24 | 21.03±1.88 | – | 25.08±4.06 | 22.66±3.05 | – |
| ALT (U/l) | 6.94±0.70a | 11.54±0.84b | 15–44<br>4–15* | 29.03±0.96a | 52.84±1.15b | 15–52<br>24–83* |
| AST (U/l) | 45.12±1.22a | 65.54±1.50b | 49–123 | 168.38±9.54a | 211.90±10.10b | 66–230 |

It is important between different letters in the same row and indicate significance $p \leq 0.05$

*Referance values*: The Merck Veterinary Manual, Ninth Edition , USA, 2005, * Altıntaş A. and UR Fidancı, 1993. A. U. Vet. Fac. Journal 40 (2): 173–186

breeds and these diseases lead to decreases in milk yields and even death at later stages (Kızıl et al., 2007; Omer et al., 2002; Nambota et al., 1994). Serum total protein, albumin, globulin and glucose concentrations decrease in animals suffering from theileriosis and it is shown that this decrease correlates with the severity of anemia (Kızıl et al., 2007). Dede et al. (2014) reported a marked decrease in glucose levels in cattle with Theileriosis. In a study conducted by Paşa et al. (2008) decreased glucose levels was demonstrated to return to normal values after treatment. Consistent with the results reported by Kızıl et al. (2007) , we found a significant decrease in serum total protein and albumin concentrations in infected animals compared with healty animals. These decreases may be associated with hipoalbunemia and hipoglobulenima. According to some studies (Ulutas et al., 2008; Fekete and Kellems, 2007) anemia, eosinophilia, and hipoproteinemia are common laboratory findings observed in gastro intestinal track nematod and liver trematoda infections. Changes in glucose levels between the groups in the current study appear to fall within normal limits.

In a study (Yadav and Sharma, 1986) serum cholesterol level was shown to be elevated in cows with theileria infection as a result of liver damage. Consistent with results obtained by Paşa et al. (2008) , cholosterol, Ca and IP levels of healthy and infected sheeps and goats were within reference values. This may be due to lack of liver damage in infected animals group.

Surgical trauma, mastitis, bacterial and parasitic infections, viral respiratory infections are known to lead to different levels of increase in serum concentration of ceruloplasmin. Ceruloplasmin is suggested to be useful marker in assessing the herd health as well as prognosis and treatment (Ulutas et al., 2008). Ulutas et al. (2008) found a higher mean levels of ceruloplasmin in diseased goats compared with the control group. Reported ceruloplasmin levels include 26.5 mg/dL (Haliloglu and Serpek, 2000), 19.3 mg/dl (Serpek et al., 1989), 13, 79 to 21, 12 mg/dl in sheep (Erdogan et al., 2003), and 21.31 mg/dl in goats (Fidancı et al., 2001). In the current study, no significant difference in ceruloplasmin levels was observed between the infected and healthy animals. This result indicates that the parasitic infections in the animals studied were not severe enough to cause an increase in ceruloplasmin, one of the positive acute phase proteins.

Increased serum ALP, ALT, AST activity levels are known to be important indicators of liver. damage (Issi et al, 2010; Ismael et al., 2013). Many studies emphasizes the formation of liver damage in theileriosis infection (Issi et al, 2010; Col and Uslu, 2007; Altug et al, 2008; Baghshani et al., 2011; Burtis et al.,2012; Basbug and Gul, 2011). In one study (Issi et al, 2010), ALT, AST and ALP activity levels were found to be elevated in infected animals with compared to control group; however, these increases were within physiological limits. There are also study (Omer et al.,2002) showing marked increases in the activites of these enzymes in infected animals which exceeded normal values. Consistent with Issi et al.(2010) , we found higher AST and ALT levels in the infected group; however, these increases appear fall within physiological limits. According to a study by Kızıl et al.(2007), increases in the activities of these enzymes in serum are associated with liver and muscle damage. Therefore, results of our study may indicate that liver and muscle damge did not exist in the infected animals included in the study.

## Conclusıon

This study is the first detailed and preliminary study of Theileria infection in sheep and goats of North Cyprus. These infections do not appear to be common in sheep and goats in Northern Cyprus. Furthermore, this study suggests that changes in some biochemical parameters may occur in infected animals that are in the early stage of disease and do not show any clinical symptoms. However, it would be useful to investigate other blood-borne diseases.

***Acknowledgements***

We thank Dean of Faculty of Veterinary Medicine, Near East University, for his support and contribution to this study.

## References

**Aktas, M., K. Altay and Dumanlı, N.**, 2005. Development of a polymerase chain reaction method for diagnosis of Babesia ovis infection in sheep and goats. *Veterinary Parasitology*, **133**: 277–281.

**Almeria, S., J. Castella, D. Ferrer, A. Ortuno, A. Estrada-Pena and J. F. Gutierrez,** 2001. Bovine piroplasm in Minorca (Balaric Island Spain): a comparison of PCR-based and light microscopy detection. *Vet. Parasitol*, **99**: 249–259.

**Altay, K., M. Aktas, N. Dumanli and M. F. Aydin,** 2008. Evaluation of a PCR and comparison with RLB for detection and differentiation of Theileria sp. MK and other Theileria and Babesia species of small ruminants. *Parasitol Res*., **103**: 319–323.

**Altuğ, N., N. Yüksek, Z. T. Ağaoğlu and I. Keleş,** 2008. Determination of adenosine deaminase activity in cattle naturally infected with *Theileria annulata*. *Trop Anim Health Prod*, 40: 449–456.

**Baghshani, H., G. R. Razmi, S. Yaghfouri and A. A. Dezaki,** 2011. Investigation of selected biochemical parameters in sheep naturally infected with theileriosis. *Comp Clin Pathol*, DOI 10.1007/s00580-011-1308-2.

**Basbug, O. and Y. Gul,** 2011. Investigation on hemolysis in cows with tropical theileriosis. *Kafkas Univ Vet Fak Derg.*, **17** (3): 421–427.

**Burtis, C.A., E. R. Ashwood and D. E. Bruns** (eds.), 2012. Tietz Textbook of Clinical Chemistry and Molecular Diagnosis (5th edition) *Elsevier, St. Louis*, USA.

**Col, R. and U. Uslu,** 2007. Changes in selected serum components in cattle naturally infected with Theileria annulata. *Bull Vet Inst Pulaw*y, **51**: 15–18.

**Dede, S., N. Altuğ, Y. Değer, N. Özdal and E. Ceylan,** 2014. Serum biochemical profile and protein fractions in cattle with theileriosis. *Revue Med. Vet.*, **165** (5-6): 137–143.

**Erdoğan, S., Z. Erdoğan and N.** Şahin, 2003. An investigation of copper, zinc,ceruloplasmin levels in serum and zinc levels in wool samples of seasonal grazing sheep. *Ankara Univ. Vet Fak Derg.,* **50:** 7–11.

**Fekete, S. G. Y. and R. O. Kellems,** 2007. Interrelationship of feding with immunity and parasitic infection a rewiew. *Veterinari Medicina*, **52**: 131–143.

**Fidancı, U. R., F. Turgay, S. Zengin, F. Kargın, S. Çelik and U. Taşdemir**, 2001. The effect of genotype on the antioxidative metabolism in angora goats. *Turk J Vet Anim Sci*, **25**: 975–981.

**Georges, K, G. R. Loria, S. Riili, A. Greco, S. Caracappa, F. Jongejan and O. Sparagano,** 2001. Detection of haemoparasites in cattle by reverse line blot hybridisation with a note on the distribution of ticks in Sicily. *Vet. Parasitol.*, **99**: 273–286.

**Haliloğlu, S. and B. Serpek,** 2000. The effect of plasma vitamin C and ceruloplasmin levels and exogen vitamin C supplementation on reproduction in sheep. *Turk J Vet Anim Sci*, **24**: 403–411.

**Inci, A., Ica, A., Yıldırım, A., Duzlu, O.**, 2010. Identification of babesia and theileria species in small ruminants in central Anatolia (Turkey) via reverse line blotting. *Turk J Vet Anim Sci*, **34** (2): 205–210.

**Irshad, N., M. Qayyum, M. Hussain and M. Q. Khan,** 2010. Prevalence of tick infestation and theileriosis in sheep and goats. *Pak Vet J*, **30** (3): 178–180.

**Ismael, A. B., A. A. Swelum, F. Khalaf and A. M. A. Abouheif,** 2013. Clinical, Haematological and Biochemical Alterations Associated with an Outbreak of Theileriosis in Dromedaries (*Camelus dromedarius*) in Saudi Arabia. *Pak Vet J*, **34** (2): 209–213.

**Issi, M., G. Yusuf, O. Başbuğ and N. Şahin,** 2010. Clinical, hematological and some biochemical parameters with serum cobalt and vitamin B12 levels in cattle with tropical theileriosis. *Kafkas Univ Vet Fak Derg.*, **16** (6): 909–913.

**Kızıl, Ö., T. Karapınar, E. Balıkçı and M. Kızıl,** 2007.Changes of heamogram and some parameters in cattle with tropical Theileriosis. http://www.fusabil.org, **21** (1): 11–14.

**Maric, V.**, 2009. Cyprus. Lonely Planet. ISBN 978-1-74104-803-2, p.229.

**Nagore, D., J. Garcia-Sanmartin, A. L. Garcia-Perez, R. A. Juste and A. Hurtado,** 2004. Identification, genetic diversity and prevalence of Theileria and Babesia species in a sheep population from Northern Spain. *Int. J. Parasito.*, **34**: 1059–1067.

**Nambota, A., K. Samui, C. Sugimoto, T. Kakuta and M. Onuma,** 1994. Theileriosis in epidemiology and Zambia : etiology, control measures. *Jpn. J. Vet. Res.*, **42** (1): 1–18.

**Omer, O. H., K. H. El-Malik and O. M. Mahmoud,** 2002. Haematological profiles in pure breed cattle naturally infected with Theileria annulata in Saudi Arabia. *Vet Parasitol*, **107**: 161–168.

**Paşa, S.**, 2008. Therapeutic efficacy of buparvaquone (buparvon) in cattle with theileriosis. *Turkish Society for Parasitology*, **32** (4): 317–321.

**Schnittger, L., H. Yin, B. Qi, M. J. Gubbels, D. Beyer, S. Niemann, F. Jongejan and J. S. Ahmed**, 2004. Simultaneous detection and differentiation of Theileria and Babesia parasites infecting small ruminants by reverse line blotting. *Parasitol. Res.*, **92**: 189–196.

**Serpek, B., N. Başpınar and S. Soysal,** 1989. The determination of serum ceruloplasmin concentration in order to Hipokuprozis diagnosis and treatment in Konya region. *I.U. Vet Fak Derg.*, **13** (1): 17–21.

**Sivakumar, T., K. Hayashida, C. Sugimoto and N. Yokoyama,** 2014. Evolution and genetic diversity of Theileria. *Infection, Genetics and Evolution*, **27**: 250–263.

**Sunderman, F. W., Jr. Nomoto and S. Nomoto,** 1970. Measurement of human serum ceruloplazmin by its p- Phenylenediamine oxidase activity. *Clinical Chemistry*, **16**: 11.

**Uilenberg, G.**, 2001. Babesiosis. In: M. W. Service (Ed.), Encyclopedia of Arthropod-Transmitted Infections of Man and Domesticated Animals. *CABI Publishing*, Wallingford, pp. 53–60.

**Ulutaş, P. A., H. Voyvoda, B. Ulutaş and S. Aypak,** 2008. Haptoglobin, serum amyloid-A and ceruloplazmin concentration in goats with mixed helminth infection. *Turkish Society for Parasitology*, **32** (4): 229–233.

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

*Received May, 13, 2015; accepted for printing February, 19, 2016*