Kafkas Univ Vet Fak Derg
19 (3): 489-493, 2013
DOI: 10.9775/kvfd.2012.8113

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Plasma Malondialdehyde, Thyroid Hormones and Some Blood Profiles in Ovine Babesiosis

Kaveh AZIMZADEH [1] Keyvan NOURI [2] Himan FAROOGHI [2]
Sohrab RASOULI [3] Negar ZAMANI [4]

[1] Department of Clinical Sciences, Veterinary Faculty, Urmia Branch, Islamic Azad University, Urmia, IRAN
[2] Graduate of Veterinary Medicine, Islamic Azad University, Urmia Branch, Urmia, IRAN
[3] Department of Pathobiology, Veterinary Faculty, Urmia Branch, Islamic Azad University, Urmia, IRAN
[4] Faculty of Science, Department of Animal Biology, Urmia University,Urmia, IRAN

Makale Kodu (Article Code): KVFD-2012-8113

Summary

Thyroid hormone and plasma malondialdehyde (MDA) levels as well as other blood parameters were performed in ovine babesiosis. The *Babesia* genus comprises pathogen parasites that cause economic problems in livestock management. Many published studies have suggested its complications, but few studies have assessed blood biochemistry. Hence, a survey of the changes in blood parameters in animals with babesiosis may be useful. Sheep with acute babesiosis were identified based on clinical signs and the observation of *Piroplasms* in red blood cells with Giemsa staining of blood smears. Blood samples were obtained from the jugular veins of 46 babesiosis-infected sheep and 46 healthy sheep without babesiosis. Malondialdehyde (MDA), Paraoxonase (PON), low density lipoprotein (LDL), high density lipoprotein (HDL), Cholestrol (Chol), Total plasma protein (TPP) and glucose (GL) in plasma and triiodothyronine (T3), thyroxine (T4) measured in serum. Levels of erythrocyte superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) and total antioxidant capacity (TAC) were also measured. The results indicated significant increases ($P<0.01$) in MDA and GL and significant decreases ($P<0.01$) in the levels of antioxidant enzymes (PON, GSH-Px, CAT, TAC [except SOD], TPP, T3, andT4, LDL, HDL, and Chol levels when compared with healthy group. The results suggested that hypothyroidism with concurrent oxidative stress are significant signs of ovine babesiosis.

Keywords: *Ovine Babesiosis, Malondialdehyde, Thyroid hormones , Blood values*

Babesiosisli Koyunlarda Plazma Malondialdehid, Tiroid Hormonları ve Bazı Kan Profilleri

Özet

Babeziyozlu koyunlarda bazı kan parametreleri ile birlikte tiroid hormonu ve plazma malondialdehit (MDA) düzeyleri incelendi. Babesia cinsindeki parazitler çiftlik hayvancılığında ekonomik sorunlara neden olurlar. *Babesia* ile ilgili yayınların çoğu hastalığın komplikasyonlarını ile ilgili olup, kan biyokimyası üzerine yapılan çalışma sayısı azdır. Bu nedenle, Babeziyozlu hayvanlarda kan parametrelerindeki değişikliklerin araştırılması yararlı olabilir. Bu çalışmadaki akut babeziyozlu koyunlar, hastalığa ait klinik belirtileri ve Giemsa ile boyanmış preparatlarda enfekte eritrositleri görerek teşhis edilmiştir. Kan örnekleri, 46 enfekte ve 46 sağlıklı koyunun juguler veninden alınmıştır. Plazmada malondialdehit (MDA), paraoksonaz (PON), LDL, HDL, kolesterol (Chol), toplam plazma protein (TPP), glikoz (GL) ve serumda triiyodotironin(T3) ve tiroksin(T4), eritrosit süperoksit dismutaz (SOD), glutatyon peroksidaz (GSH-Px), katalaz (CAT) ve total antioksidan kapasite (TAK) düzeyleri ölçülmüştür. Sağlıklı grup ile karşılaştırıldığında, enfekte hayvanlarda MDA ve GL düzeylerinde belirgin bir artış ve anti oksidant enzimleri (SOD hariç), TPP, T3, T4, LDL, HDL ve Chol düzeylerinde belirgin bir azalma görülmüştür. Bu sonuçlar hipotiroidizm ile beraber görülen oksidatif stresin, koyun babeziyozunda önemli bir gösterge olduğunu göstermektedir.

Anahtar sözcükler: *Koyun babesiosu, Malondialdehid, Tiroid hormonları, Kan profilleri*

INTRODUCTION

Babesiosis is a tick-borne hemoprotozoan disease with host-specific features carried by members of the *Babesia* genus. *Babesia ovis* induces the expression of severe pathogenic traits in sheep and causes sheep babesiosis,

İletişim (Correspondence)
+98 441 3476434
kn_az@yahoo.com

which is characterized by fever, anemia, icterus and hemoglobinuria [1,2]. *B. ovis* has been observed in most regions of Iran [1]. A high prevalence among sheep and goats in north-eastern of Iran was reported by Razmi et al.[3]. Studies of blood profiles changes in sheep babesiosis are scarce. Hematological and biochemical alterations in splenectomized sheep with experimentally induced *B. motasi* infection were investigated by Alani and Herbert [4]. In 2010 Apaydin et al.[5] investigated serum protein fractions in sheep naturally infected with babesiosis.

Anemia is a common consequence of babesiosis, which causes parasitemia and erythrocyte destruction [6]. Anemia results from the physical effects on erythrocytes, activated macrophage-mediated erythrocyte phagocytosis, antibody-affected erythrocytes and enhanced cell membrane permeability [7]. Moreover, severe cases are not always accompanied by parasitemia [6], while non-parasitic factors may also destroy erythrocytes in animals with parasite infections. Free radicals are thought to play a role in erythrocyte demolition; the assessment of free radicals in the pathogenesis of parasitic infections garnered increasing attention in recent years [7-10].

Free radicals and Reactive Oxygen Species (ROS) are continually produced during metabolic processes; levels may increase under pathological conditions and cause tissue damage [6,11]. Oxidative stress as a result of the excessive generation of free radicals, which overwhelms the antioxidants available (redox imbalance), has been reported in some animal diseases, such as pneumonia, pig's sepsis and airway disorders in horses [11-13]. Oxidative stress causes macromolecular damage, erythrocyte oxidation (cell destruction) and poly unsaturated fatty acid oxidation, which results in lipid peroxidation. Malondialdehyde (MDA) is a by-products of lipid peroxidation. The measurement of MDA levels can be used to indirectly measure the degree of lipid peroxidation and the levels of free radicals [6,9,10]. Literature regarding MDA is scarce but includes reports on elevated MDA levels in sheep naturally infected with *Babesia ovis* and cattle infected with *B. bovis* [9,14]. The thyroid gland plays a vital role in several functions, such as, oxygen consumption by cells, energy utilization, biologic processes (metabolism, growth and nervous improvement) and the productive performance of domestic animals (hair, milk, fiber). Hence, thyroid hormone levels can be investigated as markers of certain animal conditions. The thyroid hormones include of tri-iodothyronine (T3) and tetra-iodothyronine (T4). The chief secretory hormone in adult sheep is T4. Several factors influence thyroid function: e.g seasonal changes, reproductive status, age and mercury-induced hypothyroidism [15-17]. Although related research is abundant, investigations of *Babesiosis*-induced blood profile alterations are scarce.

There are no published literatures on thyroid hormones alterations in sheep babesiosis. Thus, the recent study aimed to evaluate thyroid hormones (T3, T4), MDA as a lipid peroxidation index, some blood profiles and probable interrelationship of these factors each other in ovine babesiosis.

MATERIAL and METHODS

This study was conducted in western Azerbaijan province (North west of Iran) in 2011. The animals were examined for the presence of ticks and clinical signs including hyperthermia (39.9-40.8), anorexia, petechial bleeding, icterus, and hemoglobinuria. Anemia, which is associated with pallor of the mucosal membrane, was observed in 46 sheep. Blood samples were collected via the jugular vein and blood smear staining was performed with Giemsa solution 5%. Microscopic examination in the immersion objective (X100) revealed *Piroplasms* in the same 46 sheep. The other 46 sheep without any clinical or paraclinical signs of babesiosis were selected as the healthy group. Blood samples with and without EDTA were centrifuged at 3000 RPM for 10 minutes at 4°C. MDA and PON activity were detected using the Satoh [18] and Furlong [19] method (spectrophotometer, model Cecil,Italy), respectively. T3 and T4 were measured in serum using a Roche Co. Elecsys 2010 in accordance with the electrochemiluminescence method; GL, TPP, cholesterol, HDL, and LDL (Pars azmoon Co. kits, Tehran, Iran) levels were evaluated in plasma using a Hitachi-917 Auto analyzer (Japan). SOD, GSH-Px, and TAC were measured in isolated and lysed red blood cells (by auto-analyzer, Alcyon-300, USA), (Ransod® and Ransel kits, Randox laboratories Ltd. G.B) and CAT was measured manually according to a previously described method, Abei [20]. Statistical analysis was performed for all of the data completed during the study. The Mean ± SD and the determination of variation between the data points were carried out with Student's *t*-test with SAS v9.1 (SAS Institute Inc., Cary, NC, USA). The significance level was specified at $P<0.01$.

RESULTS

All of the altered parameters are presented in *Table 1*. A significant elevation of MDA ($P<0.01$) was observed in the infected sheep compared to the healthy ones. In contrast, the level of antioxidant enzymes activity (involving CAT, GSH-Px, TAC or PON) was reduced in sheep with babesiosis compared to the healthy group ($P<0.01$). No changes in SOD activity were observed. In the case of thyroid hormones, decreases ($P<0.01$) in T3, T4, lipid profiles, and TPP were observed in infected sheep. Finally, glucose levels were increased in sheep with babesiosis as compared with the healthy group.

DISCUSSION

Lipid peroxidation plays an essential role in erythrocyte oxidation and anemia [9]. Several studies have indicated

Table 1. *Some blood parameters of sheep with babesiosis*
Tablo 1. *Babeziyozlu koyunların bazı kan parametreleri*

Parameters	Control Group	Patient Group
MDA (nmol/ml)	3.13±0.78	8.30±0.86†
T_3 (ng/ml)	1.50±0.2	0.69±0.17†
T_4 (µg/dl)	4.71±0.5	1.63±0.71†
PON (U/L)	68±12.51	26.44±4.82†
SOD (U/gHb)	714.55±74.60	678.33±90.49
CAT (k/gHb)	87.80±14.46	49.96±10.69†
GSH-Px (U/mgHb)	91.39±11.09	49.37±8.04 †
TAC (mmol/L)	0.74± 0.12	0.44±0.9†
GL (mg/dl)	57.77±7.57	93.33±10.50†
TPP (g/dl)	7.06±0.7	5.23±0.36†
Chol (mg/dl)	122.77±17.93	63.22±9.47†
HDL (mg/dl)	26.66±6.34	7.77±1.92†
LDL (mg/dl)	93.55±13.70	57.44±8†

Data are expressed as mean ± standard deviation, † Significantly different from the control group (P<0.01)

the importance of lipid peroxidation in blood parasitic diseases [6,8,10]. Various others have reported babesiosis-induced MDA elevation in domestic animals such as, sheep naturally infected with *Babesia ovis* [14]. The same authors demonstrated the important role of *B. ovis* in the induction of oxidative damage to RBCs and anemia. Moreover, Saleh [9] asserted that various causes can provoke radical species and cause lipid peroxidation in crossbred cattle naturally infected with *Babesia bigemina*: Examples include the potential role of RBCs in free- radical generation and iron`s role in sensitizing the RBC`s membrane. In this study, plasma MDA levels were found to be high in sheep with babesiosis. The likely cause of this elevation is the over-production of free radicals (ROS) and their induced lipid peroxidation. All of the above- mentioned findings are in accordance with the findings presented here. One of the other probable causes of MDA enhancement might be associated with hypothyroidism and increasing in lipid peroxidation, as documented in rats and humans. However, no association between MDA elevation and hypothyroidism has been observed in sheep. Erdamar et al. [21] demonstrated MDA elevation and lipid peroxidation in overt hypothyroidism and hyperthyroidism and concluded that thyroid hormones play an important role in oxidative stress. Moreover, Cano-Europa [22] reported a correlation between thyroid dysfunction and the induction of selective oxidative stress in the rat amygdala and hippocampus. Other authors have reported increases in oxidative stress in the context of hypothyroidism [23,24]. In this report, we observed decreases in T3 and T4. The following factors may influence hypothyroidism-induced lipid peroxidation in sheep: 1) Increased production of hydrogen peroxide (H_2O_2) via low levels of thyroid hormones through the over-activity of superoxide dismutase (manganese dependent) and a decrease in catalase activity in hepatocyte mitochondria [22]. 2) A decline in Na^+/K^+ ATPase activity in the cell membrane. Pachcco-Rosado et al.[25] and Carageorgiou et al.[26] described a decrease in hippocampus Na^+/K^+ ATPase activity in hypothyroid rats and Yur et al.[2] reported Na^+/K^+ ATPase activity decreases in sheep naturally infected with babesiosis. It is likely that decreased levels of Na^+/K^+ ATPase activity would have been observed in the animals studied here, eventually resulting in lipid peroxidation via unknown mechanism(s).

Several studies demonstrated diminishing of antioxidant enzymes (SOD, CAT, GSH-Px, TAC) in hemoprotozoan diseases in domestic animals [14,30] which is in accordance with our study except for SOD activity. Chaudhuri et al.[6] reported a significant elevation of CAT and SOD in canine babesiosis compared with non-babesiosis dogs, which is also in contrast to the results presented here.

PON is a hydrolase that plays a major role in xenobiotic metabolism such as involving in the detoxification of organophosphorus compounds and inhibiting lipid peroxidation [31,32]. There are many reports about PON activity in humans, but reports in the context of veterinary medicine are scarce [31,33]. PON levels were found to be low in this study. The following represent potential reasons for the decrease: 1) A protective role of PON in the peroxidation of lipids and inhibition of the harmful effects of MDA. 2) Decreased HDL levels. Various studies have reported an association between the N-terminal of paraoxonase and apolipoprotein A1 (Apo A1) of HDL [31]. Hence, a decrease in PON might be simultaneously correlated with HDL decreases.

Hypothyroidism is the most prevalent thyroid disturbances in small ruminants and causes disorders that reduce the animal's ability to defend against infections and render it vulnerable to ketosis [34]. Diverse factors such as, iodine deficiency and chronic disease can induce hypothyroidism [15]. Certain situations may cause hypothyroidism without clinical signs, as is typical of secondary euthyroid conditions: for example, Pituitary-Thyroid(HPT) axis disorders and the possible effects on hormone- binding proteins in serum, disturbances in hormone metabolism, disorders in the peripheral distribution of hormones, the inhibition of hormone synthesis and an increase in the release of cytokines (e.g., interleukin-1, interleukin-2, interferon gamma and tumor necrosis factor α) that may be caused anorexia as well as the inhibition of secretion by the glandular portion of the thyroid [35]. Issi et al.[36] reported low levels of free T3, free T4, total T3 and total T4 in the pre-treatment of cattle naturally infected with *Theileria annulata*. A similar study performed by Badiei et al.[37] in crossbred Holstein cattle naturally infected with *Theileria annulata* revealed low levels of T3, T4, free T3 and free T4. The authors postulated a paramount effect of anorexia.

Kumar et al.[35] reported decreases in T3 and T4 in dogs with concurrent *Erlichia canis* and *Babesia gibsoni* infections and hypothesized that the body adapts by decreasing cellular metabolism during the period of disease. In recent

studies the levels of T3 and T4 were low. It is likely that certain factors have induced diminished levels in sheep with bebesiosis. These factors include low iodine levels in soil and food, cytokines elevations due to disease that may cause anorexia and thyroid activity suppression, deficiencies of trace elements (e.g selenium [37], and bodily adaptations such as decreased cellular metabolism during periods of disease as well as seasonally induced alterations [35,38].

Thyroid hormones influence lipid and lipoprotein metabolism in diverse species such as sheep, goat, horse and camel. Moreover, thyroid hormones increase lipolysis in adipose tissue and stimulate lipogenesis and the associated enzyme activity. The relationship between thyroid hormones and cholesterol remains to be elucidated. Notably, triglyceride levels do not correlate with cholesterol or thyroid hormone levels in camels and goats [30,38,39]. Bartley [40] reported cholesterol decreases resulting from the catabolic effect of liver mediated by thyroid hormones. Ibrahim et al.[41] demonstrated the inverse correlation of triglycerides, cholesterol and phospholipid concentrations with hyperthyroidism in stricken Nubian goats. Santi [42] reported an inverse association between hypothyroidism and total cholesterol, triglyceride and LDL -cholesterol. Conflicting data exist regarding the relationship between thyroid function and cholesterol and triglyceride levels. We observed decreases in all aspects of the lipid profile, as reported by Bartley [40]. Various factors may have affected this decrease, such as hypothyroidism, malnutrition and /or seasonal effects.

Thyroid hormones are considerable mediators of glucose metabolism and their roles in provoking glucose utilization have been established [43,44]. Forhead et al.[45] described the effects of thyroid hormones in decreasing liver glycogen storage during late pregnancy in the ovine fetus and suggested a possible influence of hypothyroidism and the related difficulties with euglycemia regulation. Keller et al.[46] reported hypoglycemia in many dogs that suffered from babesiosis and indicated the importance of prompt hypoglycemia detection in canine babesiosis. The significant increase in glucose levels in these animals compared with the non-babesiosis group observed in this study may have been due to disease - the stress induced and/or intravascular hemolysis [47].

Infected animals exhibited a decrease in TPP when compared to healthy ones. Several studies have demonstrated TPP decreases in dog and sheep with babesiosis [5,10,48,49]. Moreover, Barrera et al.[50] suggested that TPP decreases may be due to anorexia in horse babesiosis. Our results generally support this assertion.

In conclusion, on the basis of similar published studies in various species of domestic animals, the results presented here suggest that oxidative stress is a common complication of ovine babesiosis. Moreover, low levels of T3 and T4, which denote hypothyroidism may exacerbate oxidative stress. Thus, it can be concluded that the concurrent occurrence of oxidative stress and hypothyroidism may negatively impact farm animals and thereby meat or dairy production.

REFERENCES

1. Rahbari S, Nabian S, Khaki Z, Alidadi N, Ashrafihelan J: Clinical, haematologic and pathologic aspects of experimental ovine babesiosis in Iran. *Iran J Vet Res,* 9, 59-64, 2008.

2. Yur F, Yazar M, Değer Y, Dede S: Na+/K+ ATPase activity in sheep with natural babesiosis. *Acta Vet Brno,* 79, 233-236, 2010.

3. Razmi GR, Naghibi A, Aslani MR, Dastjerdi K, Hosseini H: An epidemiological study on *Babesia* infection in small ruminants in Mashhad suburb, Khorasan province, Iran. *Small Rumin Res,* 50, 39-44, 2003.

4. Alani AJ, Herbert IV: The pathogenesis of *Babesia motasi* (Wales) infection in sheep. *Vet Parasitol*, 27, 209-220, 1988.

5. Apaydin B, Dede S: Electrophoretic profile of serum protein fractions from sheep naturally infected with *Babesia ovis. Rev Med Vet*, 161, 57-60, 2010.

6. Chaudhuri S, Varshney JP, Patra RC: Erythrocytic antioxidant defense, lipid peroxides level and blood iron, zinc and copper concentrations in dogs naturally infected with *Babesia gibsoni. Res Vet Sci,* 85, 120-124, 2008.

7. Saleh MA: Erythrocytic oxidative damage in crossbred cattle naturally infected with *Babesia bigemina. Res Vet Sci,* 86, 43-48, 2009.

8. Kumar A, Varshney JP, Patra RC: A comparative study on oxidative stress in dogs infected with *Ehrlichia canis* with or without concurrent infection with *Babesia gibsoni. Vet Res Commun*, 30, 917-920, 2006.

9. Saleh MA: Circulating oxidative stress status in desert sheep naturally infected with *Fasciola hepatica. Vet Parasitol*, 154, 262–269, 2008a.

10. Deger S, Deger Y, Bicek K, Ozdal N, Gul A: Status of lipid peroxidation, antioxidants, and oxidation products of nitric oxide in equine Babesiosis: Status of antioxidant and oxidant in equine babesiosis. *J Equine Vet Sci,* 29, 743-747, 2009.

11. Castillo C, Hernandez H, Bravo A, Lopez-Alonso M, Pereira V, Benedito JL: Oxidative status during late pregnancy and early lactation in dairy cows. *Vet J,* 169, 286-292, 2005.

12. Hata K, Kaneda T: Effect of autoxidized oil on carp. *Bull Japan Soc Sci Fish,* 46, 997-1000, 1980.

13. Lykkesfeldt J, Svendsen O: Oxidants and antioxidants in disease: Oxidative stress in farm animals. *Vet J*, 173, 502-511, 2007.

14. Esmaeilnejad B, Tavassoli M, Asri-Rezaei S, Dalir-Naghadeh B: Evaluation of antioxidant status and oxidative stress in sheep naturally infected with *Babesia ovis. Vet Parasitol*, doi:10.1016/j.vetpar, 2011.

15. Todini L: Thyroid hormones in small ruminants: Effects of endogenous, environmental and nutritional factors. *Small Ruminant Res,* 68, 285-290. 2007.

16. Novoselec J, Antunović Z, Šperanda M, Steiner Z, Šperanda T: Changes of thyroid hormones concentration in blood of sheep depending on age and reproductive status. *Ital J Anim Sci,* 8, 208-210, 2009.

17. Badiei KH, Mostaghni KH, Nikghadam P, Pourjafar M: The effect of mercury on thyroid function in sheep. *Int J Vet Res,* 4, 277-281, 2010.

18. Satoh K: Serum lipid peroxide in cerebrovascular disorders determined by new colorimetric method. *J Clin Chem,* 90, 37-43, 1978.

19. Furlong CE, Richter RJ, Seidel SL, Motulsky AG: Role of genetic polymorphism of human plasma paraoxonase/arylesterase in hydrolysis of the metabolites chlorpyrifos oxon and paraoxon. *Am J Hum Genet,* 43, 230-238, 1988.

20. Abei H: Catalase *in vitro*. *Methods in Enzymology,* 105, 121-126, 1984.

21. Erdamar H, Demirci H, Yaman H, Kemal Erbil M, Yakar T, Sancak B, Elbeg S, Biberoglu G, Yetkin I: The effect of hypothyroidism, hyperthyroidism, and their treatment on parameters of oxidative stress and antioxidant status. *Clin Chem Lab Med*, 46, 1004-1010, 2008.

22. Cano-Europa E, PCrez-Severiano F, Vergara P, Ortiz-Butron R, Rios C, Segovia J, Pacheco-Rosado J: Hypothyroidism induces selective oxidative stress in amygdala and hippocampus of rat. *Metab Brain Dis,* 23,

275-287, 2008.

23. Fernandez V, Tapia G, Varela P, Romanque P, Cartier-Ugarte D, Videla LA: Thyroid hormone-induced oxidative stress in rodents and humans: A comparative view and relation to redox regulation of gene expression. *Comp Biochem Phys,* 142, 231-239, 2006.

24. Babu K, Jayaraaj IA, Prabhakar J: Effect of abnormal thyroid hormone changes in lipid peroxidation and antioxidant imbalance in hypothyroid and hyperthyroid patients. *Int J Biol Med Res,* 2, 1122-1126, 2011.

25. Pacheco-Rosado I, Arias-Citalan G, Ortiz-Butron R, Rodriquez-Paez L: Selective decrease of Na^+K^+-ATPase activity in brain of mild hypothyroid rats. *Proc West Pharmacol Soc,* 48, 52-54, 2005.

26. Carageorgiou H, Pantos C, Zarros A, Stokakis V, Mourouzis I, Cokkinos D, Tsakiris S: Changes in acetylcholinesterase, Na^+/ K^+-ATPase and Mg^{2+} -ATPase activities in the frontal cortex and the hippocampus of hyper- and hypothyroid adult rats. *Metab Clin Exp,* 56, 1104-1110, 2007.

27. Amato A, Barbour B, Szatkowski M, Attwell D: Counter-transport of potassium by glutamate uptake carrier in glial cell isolated from the tiger salamander retina. *J Physiol (Lond)*, 479, 371-390, 1994.

28. Rossi DJ, Oshima T, Attvell D: Glutamate release in severe brain ischaemia is mainly by reversed uptake. *Nature,* 403, 316-321, 2000.

29. Matsunaga M, Saotanie M, Satoh H, Katoh H, Terrade H, Ilayashi H: Different actions of cardioprotective agents on mitochondrial Ca^{2+} paradox-induced Ca^{2+} overload. *Circ J,* 69, 1132-1140, 2005.

30. Nazifi S, Razavi SM, Kianiamin P, Rakhshandehroo E: Evaluation of erythrocyte antioxidant mechanisms: Antioxidant enzymes, lipid peroxidation, and serum trace elements associated with progressive anemia in ovine malignant theileriosis. *Parasitol Res,*109, 275-281, 2011.

31. Turk R, Juretic D, Geres D, Turk N, Rekic B, Simeon-Rudolf V, Svetina A: Serum paraoxonase activity and lipid parameters in the early postpartum period of dairy cows. *Res Vet Sci,* 76, 57-61, 2004.

32. Aviram M, Rosenblat M: Paraoxonase and cardiovascular diseases: Pharmacological and nutritional influences. *Curr Opin Lipidol,* 16, 393-399, 2005.

33. Ece A, Atamer F, Gurkan M, Davutoglu M, Bilici M, Tutan M, Gunes A: Paraoxonase, anti-oxidant response and oxidative stress in children with chronic renal failure. *Pediatrics Nephrology*, 21, 239-245, 2006.

34. Gupta B, Moolchandani A, Sareen M: Effect of induced Hypothyroidism on plasma cholesterol and bilirubin in Marwari Sheep. *Vet World,* 3, 323-325, 2010.

35. Kumar A, Varshney JP, Varshney VP: Endocrine dysfunction in chronic severe ehrlichiosis with or without babesiosis in dogs. *Vet Res Commun*, 30, 911-916, 2006.

36. Issi M, Gul Y, Basbug O: The effect of classical theileriosis treatment on thyroid hormones levels in cattle naturally infected *Theileria annulata*. *Asian J Anim Vet Adv,* 6, 531-536, 2011.

37. Badiei Kh, Mostaghni Kh, Pourjafar M, Ghane M, Mohammadi E: Serum thyroid hormones and trace element concentrations in crossbred Holstein cattle naturally infected with *Theileria annulata*. *Comp Clin Pathol*, DOI 10.1007/s00580-010-0962-0, 2010b.

38. Nazifi S, Saeb M, Rowghani E, Hasankhani M, Hasanshahi F, Gafari N: Studies on the physiological relationship between thyroid hormones, serum lipid profile and erythrocyte antioxidant enzymes in clinically healthy Iranian fat tailed sheep. *Bulg J Vet Med,* 10, 161-167, 2007.

39. Tajik J, Nazifi S: Serum concentrations of lipids and lipoproteins and their correlations together and with thyroid hormones in Iranian water buffalo *(Bulbalus bulbalis). Asian J Anim Vet Adv,* 5, 196-201, 2011.

40. Bartley JC: Clinical Biochemistry of Domestic Animals. pp. 106-141, Academic Press, New York, 1994.

41. Ibrahim RE, Maglad MA, Adam SE, Mirghani TE, Wasfi IA: The effect of altered thyroid status on lipid metabolism in Nubian goats. *Comp Biochem Phys*, 77, 507-512, 1984.

42. Santi A: Association between thyroid hormones, lipids and oxidative stress biomarkers in overt hypothyroidism. *Clin Chem Lab Med,* 48, 1635-1639, 2010.

43. Fowden AL, Silver M, The effects of thyroid hormones on oxygen and glucose metabolism in the sheep fetus during late gestation. *J Physiol,* 482, 203-213, 1995.

44. Boelen A: Thyroid hormones and glucose metabolism: the story begins before birth. *Exp Physiol* , DOI: 10.1113/expphysiol.049361, 2009.

45. Forhead AJ, Cutts S, Matthews PA, Fowden AL: Role of thyroid hormones in the developmental control of tissue glycogen in fetal sheep near term. *Exp Physiol,* 94, 1079-1087, 2009.

46. Keller N, Jacobson LS, Nel M, Clerq MD, Thompson PN, Schoeman JP: Prevalence and risk factors of hypoglycemia in virulent canine babesiosis. *J Vet Intern Med,* 18, 265-270, 2004.

47. Lakshmi NR, Sreedevi C, Annapurna P, Aswani KK: Clinical management and hemato-biochemical changes in babesiosis in buffaloes. *Buffalo Bull,* 29, 92-94, 2010.

48. Camacho AT, Guitian FJ, Palas E: Serum protein response and renal failure in canine Babesia annae infection. *Vet Res,* 36, 713-722, 2005.

49. Lobetti RG, Mohr AJ, Dippenaar T, Myburgh E: A preliminary study on the serum protein in canine Babesiosis. *J S Afr Vet Assoc,* 71, 38-40, 2000.

50. Barrera R, Carapeto MV, Habela MA, Zaragoza C: Electrophoretic pattern of serum proteins in horses with babesiosis. *Arch Med Vet,* 42, 173-178, 2010.