RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES
PRINT ISSN 2221-1896, ONLINE ISSN 2223-0343
www.roavs.com

Status of some oxidative stress biomarkers in sheep naturally infected with theileriosis

Hasan Baghshani[1], Gholam Reza Razmi[2], Saeed Yaghfouri[2] and Amin Ahmadi Dezaki[2]

[1]Department of Basic Sciences, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
[2]Department of Pathobiology, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Abstract

The present study was aimed to investigate some oxidative stress indices in the erythrocytes from sheep naturally infected with theileriosis. Thirty adult fat-tailed sheep suffering from theileriosis were selected on the basis of clinical examination and positive peripheral blood smears and twenty clinically healthy animals without parasitaemia (according to the blood smears) served as controls. The oxidative stress indices including enzymatic activities of superoxide dismutase, glutathione peroxidase and catalase, glutathione and malondialdehyde were measured in erythrocyte haemolysate. The activities of antioxidant enzymes including superoxide dismutase and glutathione peroxidase were considerably decreased in infected sheep compared to controls, although the decrease was only significant ($P<0.05$) for superoxide dismutase activity. By contrast, catalase activity was non-significantly higher in the infected group compared to control group. Malondialdehyde concentration was significantly enhanced in erythrocytes of infected group, whereas glutathione level was significantly reduced in infected group in comparison to controls. Disturbed antioxidant mechanisms of erythrocytes in infected group, accompanied by a significant rise in erythrocytic lipid peroxidation, implied that the oxidative stress may have a pathophysiological role in ovine theileriosis.

Keywords: Theileriosis, sheep, oxidative stress, endogenous antioxidants

Introduction

Theileriosis is a tick-borne infectious, haemoprotozoan disease of wild and domestic ruminants in the tropical and subtropical regions of the world, caused by the genus *Theileria*. It causes serious economic losses through mortality and loss of productivity (Glass et al., 2003). *Theileria* spp. are intracellular parasites that complete their life cycle in the mammalian hosts by successively utilizing lymphoid cells and erythrocytes (Soulsby, 1982). *T. lestoquardi* and *T. ovis* are suspected to cause ovine theileriosis in Iran (Hashemi-Fesharaki, 1997).

Oxidative stress resulting from increased production of free radicals and reactive oxygen species (ROS), and/or a decrease in antioxidant defence, leads in impairment of DNA, enzymes and membranes and induces changes in the activity of the immune system and in the structure of basic biopolymers which, in turn, may be related to various health disorders (Trevisan et al., 2001; Abd Ellah, 2010). In a number of studies, the amount of reactive oxygen radicals increased in cells of hosts infected with different species of parasites (Abd Ellah, 2010). Oxidative stress has been reported in some haemoparasitic diseases of animals such as theileriosis in cattle, buffaloes and sheep (Sahoo et al., 2001; Shiono et al., 2003; Grewal et al., 2005; El-Deeb and Younis, 2009; Nazifi et al., 2011), babesiosis in horses and cattle (Deger et al., 2009; Saleh, 2009) and trypanosomiasis in camels (Saleh et al., 2009). There are some evidences that oxidative damage to cellular components incorporate in pathogenesis of anaemia in theileriosis (Asri Rezaei and Dalir-Naghadeh, 2006; Nazifi et al., 2011). On the other hand, the understanding of the pathophysiology of oxidative stress in theileriosis will allow the design of specific antioxidant therapies.

The present study was conducted to assess the pattern of changes and the relative values of some endogenous antioxidants and the level of malondialdehyde, as a biomarker of lipid peroxidation, in erythrocytes of sheep naturally infected with theileriosis. These studies may be of value for further understanding the pathogenesis of the disease and as an aid in diagnosis and supportive therapy of ovine theileriosis.

Materials and Methods

Thirty adult fat-tailed sheep suffering from ovine theileriosis were selected from some farms in Fasa district, Iran, during the peak months of theileriosis occurrence in 2010. Infected sheep were selected on the basis of clinical examination and positive peripheral blood smears. Thin blood smears were prepared from the ear vein, and stained with Giemsa for confirmation of the disease on the basis of microscopical observation of piroplasms of *Theileria* in erythrocytes. On the other hand, 20 clinically healthy animals without parasitaemia located in the same farms served as controls. All sheep included in the study ranged from 2 to 4 years old of both sexes.

Blood samples were collected by venepuncture into EDTA-containing tubes from all infected sheep and controls. The blood tubes were placed on ice until laboratory arrival (<2 h). The samples were centrifuged at 750 g for 20 min, and after plasma separation erythrocyte pellet was washed three times with normal saline solution. The washed centrifuged erythrocytes were haemolysed by the addition of an equal volume of ice-cold redistilled water and prepared haemolysate were stored at -70°C until analysis.

Glutathione peroxidase (GPx) activity was measured by the method of Paglia and Valentine (1967) using RANDOX-Ransel enzyme kit. In this method, GPx catalyzes the oxidation of GSH by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form, with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm was measured spectrophotometrically, and the results were expressed as units per gram haemoglobin. Haemoglobin (Hb) concentration was measured by cyanmethaemoglobin method.

Superoxide dismutase (SOD) activity was determined by a modified method of iodophenyl nitrophenol phenyltetrazolium chloride using the RANDOX-Ransod enzyme kit. This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophend)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The SOD activity was then measured by the degree of inhibition of this reaction. One unit of SOD was considered a 50% inhibition of reduction of INT under the condition of the assay. The results were expressed as units per gram haemoglobin.

Catalase (CAT) activity was measured in the RBC haemolysate by the method described by Claiborne (1986) and expressed as units per gram haemoglobin. The decomposition of H_2O_2 can be directly followed by the decrease of absorbance at 240 nm. The difference in absorbance at 240 nm per time unit allows determining the CAT activity.

Non-enzymatic antioxidant, reduced glutathione (GSH), was assayed by the method previously described by Ellman (1959). In this method, 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) is reduced to 2-nitro-5′-mercaptobenzoic acid (NMBA) by GSH. The amount of the yellow colour reduced was measured at 412 nm and expressed as micromoles per gram haemoglobin.

Determination of malondialdehyde (MDA) concentration in erythrocyte haemolysate was performed using the method of thiobarbituric acid which measures MDA-reactive products (Placer et al., 1966), as described by Todorova et al. (2005). The concentration of MDA was calculated using a molar extinction coefficient value of 156,000 M^{-1} cm^{-1}. The results were expressed as nanomoles of MDA per gram haemoglobin.

Statistical analysis

All experimental values were presented as mean ± standard error of mean (SEM). The obtained data were analyzed using Student's t-test. The level of significance was set at $P<0.05$. All calculations were performed using SPSS/PC software.

Results

The values (mean ± SEM) of the measured erythrocytic oxidative stress parameters in healthy and affected sheep are presented in Table 1. The activities of antioxidant enzymes including GPx and SOD were considerably decreased in infected sheep compared to controls, although the decrease was only significant ($P < 0.05$) for SOD activity. By contrast, CAT activity was non-significantly higher in the infected group compared to control group.

As shown in Table 1, MDA concentration was significantly enhanced in erythrocyte haemolysate of infected group whereas GSH level was significantly reduced in infected group in comparison to healthy controls.

Discussion

Oxidative stress is an active field of research in ruminant medicine and has been implicated in numerous disease processes. Although the study of oxidative stress is a relatively young field of research in ruminant medicine, the understanding of the role of oxidants and antioxidants in physiological and pathological conditions is rapidly increasing (Celi, 2011). In this study on ovine theileriosis, our results

Table 1: Erythrocytic superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) activities, malondialdehyde (MDA) level and glutathione (GSH) content in sheep naturally infected with theileriosis.

Parameter	Uninfected sheep (n= 20)	Infected sheep (n=30)	Change %
GPx (U/g Hb)	277.00±47.26	223.20±32.47	-19.42
SOD (U/g Hb)	1022.35±93.10	794.97±59.36*	-22.24
CAT (U/g Hb)	298.38±34.76	378.00±33.53	26.68
GSH (µmol/g Hb)	6.30±0.61	4.20±0.51*	-33.33
MDA (nmol/g Hb)	98.80±15.38	188.43±31.56*	90.72

*significantly different at $P<0.05$

indicate that infection with *Theileria* led to notable changes in measured oxidative stress indices.

Assaying antioxidant enzymes is among the most widely used methods for determination of oxidative stress. The seleno-enzyme glutathione peroxidase contributes to the oxidative defense of animal tissues by catalyzing the reduction of hydrogen and lipid peroxides (Arthur, 2000). GPx functions in cellular oxidation-reduction reactions to protect the cell membrane from oxidative damage caused by free radicals (Flohe et al., 1973). Various responses of GPx activity in *Theileria* infected ruminants has been reported by various authors. Naziroglu et al. (1999) showed that GPx activities in plasma and erythrocytes did not differ significantly between a group of cattle naturally infected with *T. annulata* and treated with buparvaquone and another group of uninfected untreated controls. Grewal et al. (2005) reported that GPx activity exhibits a significant rise in cattle naturally infected with *T. annulata*. On the other hand, Asri-Rezaei and Dalir-Naghadeh (2006), El-Deeb and Younis (2009) and Nazifi et al. (2011) reported significant decrease in GPx activity in *Theileria* infected cattle, buffaloes and sheep, respectively. Moreover, our results indicated a non-significant decrease in erythrocytic GPx activity in *Theilria* infected sheep in comparison to controls.

Catalase is of equal importance to GPx in the defence of human erythrocytes against H_2O_2 generating reactions (Harvey, 1997). According to the results of this study, catalase levels were slightly increased in affected sheep. The obtained results were in line with that reported by Asri-Rezaei and Dalir-Naghadeh (2006) who reported significant increase in the activity of CAT in *Theileria* infected cattle with mild or moderate anaemia in comparison to healthy cattle and infected cattle with severe anaemia. Increased activity of catalase had been also reported in *Babesia* infected dogs (Chaudhuri et al., 2008) and *Trypanosoma* infected camels (Saleh et al., 2009). On the other hand, while Grewal et al. (2005) reported no substantial changes in the CAT activity in *Theileria* infected cattle, El-Deeb and Younis (2009) and Nazifi et al. (2011) reported significant decrease in the CAT activity in *Theileria* infected buffaloes and sheep, respectively.

Superoxide dismutase is important in the antioxidative defence mechanism and protects against lipid peroxidation (Halliwell and Chirico, 1993; Miller et al., 1993). According to our data, erythrocytic SOD activity in *Theileria* infected sheep was significantly lower than the parasitologically free controls. Similar findings had been reported in bovine (Asri-Rezaei and Dalir-Naghadeh, 2006; El-Deeb and Younis, 2009) and ovine theileriosis (Nazifi et al., 2011). Decreased erythrocytic SOD activity has been reported in *Trypanosoma* infection in humans (Wen et al., 2004) and camels (Saleh et al., 2009) and *Plasmodium* infection in humans (Erel et al., 1997). However, the obtained results were different from that reported by Grewal et al. (2005) who reported no substantial changes in the activity of SOD in cattle naturally infected with *Theileria*. In addition significant increases in erythrocytic superoxide dismutase activity have been reported in babesiosis in dogs (Chaudhuri et al., 2008) and anaplasmosis in cattle (Nazifi et al., 2008).

Both increased and decreased antioxidant enzyme levels have been reported in different conditions as a consequence of enhanced ROS production either by up-regulation of enzyme activity or utilization of the antioxidant enzymes to counter the ROS. Considering the fact that mature red cells lack protein synthesis machinery and cannot replace damaged proteins, it can be assumed that SOD and GPx are consumed to scavenge ROS and their decline in infected animals may be due to degradation by ROS during the detoxifying process. Since both CAT and GPX are responsible for enzymatic removal of H_2O_2, the higher activity of CAT in infected group may provide some extra protection against H_2O_2 to increase the lifespan of erythrocytes. Non-significant increase in CAT activity might be due to increased specific activity of pre-existing enzyme as an indirect compensatory response to increased oxidant challenge.

GSH is required for the disposal of H_2O_2 by the reaction catalysed by GPx. Depletion of tissue GSH is one of the primary factors that permit lipid peroxidation (Konukoglu et al., 1998). The significant reduction in GSH amount was in line with the findings of El-Deeb and Younis (2009). They reported a significant reduction in the levels of glutathione in *T. annulata*

infected buffaloes compared with healthy buffaloes. It was reported also that babesiosis in sheep (Bicek et al., 2005) and horse (Deger et al., 2009) and trypanosomiasis in camel (Saleh et al., 2009) can lead to significant reduction in GSH levels, thus supporting the findings of this study.

Among the known biological molecules, lipids are one of the most susceptible substrates to free radicals damage and biomarkers of lipid peroxidation are considered the best indicators of oxidative stress (Georgieva, 2005). Lipid peroxidation is a non-enzymatic chain reaction based on oxidation of mainly unsaturated fatty acids and is associated with the presence of reactive oxygen species. Malondialdehyde is one of the end-products of lipid peroxidation, and the extent of lipid peroxidation is most frequently measured by estimating MDA levels (Lata et al., 2004). Several lines of evidence have suggested an important role for Lipid peroxidation in the pathogenesis of several parasitic diseases (Bagchi et al., 1993; Deger et al., 2009). Based on the present results, a significant increase of MDA concentration was found in the erythrocytes of *Theileria* infected sheep in comparison to the control group that indicate a high production of free radicals in diseased sheep. Our finding of increase in erythrocytic MDA is consistent with the results of Naziroglu et al. (1999), Sahoo et al. (2001), Grewal et al. (2005), Asri-Rezaei and Dalir-Naghadeh (2006) and El-Deeb and Younis (2009), who observed an increase of MDA in red blood cells in bovine theileriosis. Similar finding had been reported by Nazifi et al. (2011) who reported the increased levels of MDA in ovine theileriosis. Also there are several reports that infection with some other haemoparasites is associated with a marked elevation in lipid peroxidation (Murase et al., 1996; Griffiths et al., 2001; Eze et al., 2008; Chaudhuri et al., 2008; Deger et al., 2009; Saleh, 2009; Saleh et al., 2009). The increased MDA content observed in this study may not only be due to increased free radical generation but also be exacerbated by the inefficient endogenous antioxidant capacity.

Oxidative injury to the erythrocyte membrane is evidenced by increased formation of MDA during progress of infection. The erythrocytes membrane is rich in polyunsaturated fatty acids, a primary target for reactions involving free radicals (May et al., 1998; Devasena et al., 2001). Moreover, repeated exposure to high concentration of oxygen or presence of iron renders erythrocytes highly susceptible to peroxidative damage (Clemens and Waller, 1987). The higher production of ROS and consequent elevated lipid peroxide concentrations renders the erythrocytes more fragile and prone to lysis which might has an important role in the pathogenesis of anaemia in case of theileriosis (Grewal et al., 2005; Asri-Rezaei and Dalir-Naghadeh, 2006).

In conclusion, the glutathione level and the activities of measured antioxidant enzymes excluding CAT have been shown to be reduced which indicates that erythrocytes have depleted antioxidant mechanisms in a response to *Theileria* infection in sheep. Disturbed antioxidant mechanisms of erythrocytes, accompanied by a significant rise in lipid peroxidation of erythrocytes, implied that the oxidative stress may have a pathophysiological role in ovine theileriosis.

Acknowledgement

This research was financially supported by grant (NO. 3444) from Ferdowsi University of Mashhad, Mashhad, Iran.

References

Abd Ellah, M.R. 2010. Involvement of free radicals in animal diseases. *Comparative Clinical Pathology*, 19: 615–619.

Arthur, J.R. 2000. The glutathione peroxidases. *Cellular and Molecular Life Sciences*, 57: 1825–1835.

Asri Rezaei, S. and Dalir-Naghadeh, B. 2006. Evaluation of antioxidant status and oxidative stress in cattle infected with *Theileria annulata*. *Veterinary Parasitology*, 142: 179–186.

Bagchi, M., Mukherjee, S. and Basu, M.K. 1993. Lipid peroxidation in hepatic microsomal membranes isolated from mice in health and in experimental leishmaniasis. *Indian Journal of Biochemistry and Biophysics*, 30: 277–281.

Bicek, K., Deger, Y. and Deger, S. 2005. Some biochemical and haematological parameters of sheep infected with *Babesia* species.*Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi*, 16: 33–35.

Celi, P. 2011. Oxidative stress in ruminants. In: Mandelker, L. and Vajdovich, P. (editors), Studies on veterinary medicine, oxidative stress in applied basic research and clinical practice. 1st (ed.), Humana Press, Pp: 191–231.

Chaudhuri, S., Varshney, J.P. and Patra, R.C. 2008. Erythrocytic antioxidant defense, lipid peroxides level and blood iron, zinc and copper concentrations in dogs naturally infected with *Babesia gibsoni*. *Research in Veterinary Science*, 85: 120–124.

Claiborne, A. 1986. Catalase activity. In: Greenwald, R.A. (editor), CRC handbook of methods for oxygen radical research. Boca Raton., FL: CRC Press, Pp: 283–284.

Clemens, M.R. and Waller, H.D. 1987. Lipid peroxidation in erythrocytes. *Chemistry and Physics of Lipids*, 45: 251–268.

Deger, S., Deger, Y., Bicek, K., Ozdal, N. and Gul, A. 2009. Status of lipid peroxidation, antioxidants, and oxidation products of nitric oxide in equine babesiosis: status of antioxidant and oxidant in equine babesiosis. *The Journal of Equine Veterinary Science*, 29 (10): 743–747.

Devasena, T., lalith, S. and Padma, K. 2001. Lipid peroxidation, osmotic fragility and antioxidant status in children with acute post-streptococcal glomerulonephritis. *Clinica Chimica Acta*, 308: 155–161.

deZwart, L.L., Meerman, J.H.N., Commandeur, J.N.M. and Vermeulen, N.P.E. 1999. Biomarkers of free radical damage. Applications in experimental animals and in humans. *Free Radical Biology and Medicine*, 26: 202–226.

El-Deeb, W.M. and Younis, E.E. 2009. Clinical and biochemical studies on *Theileria annulata* in Egyptian buffaloes (*Bubalus bubalis*) with particular orientation to oxidative stress and ketosis relationship. *Veterinary Parasitology*, 164: 301–305.

Ellman, G.L .1959. Tissue sulphydryl group. *Archives of Biochemistry and Biophysics*, 82: 70–77.

Erel, O., Kocyigit, A., Avci, S., Aktepe, N. and Bulut, V. 1997. Oxidative stress and antioxidative status of plasma and erythrocytes in patients with vivax malaria. *Clinical Biochemistry*, 30: 631–639.

Eze, J.I., Anene, B.M. and Chukwu, C.C. 2008. Determination of serum and organ malondialdehyde (MDA) concentration, a lipid peroxidation index, in *Trypanosoma brucei*-infected rats. *Comparative Clinical Pathology*, 17: 67–72.

Flohe, L., Gunzler, W.A. and Schock, H.H. 1973. Glutathione peroxidase: a selenoenzyme. *FEBS Letters*, 32:132–134.

Georgieva, N.V. 2005. Oxidative stress as a factor of disrupted ecological oxidative balance in biological systems–a review. *Bulgarian Journal of Veterinary Medicine*, 8: 1–11.

Glass, E.J., Craigmile, S.C., Springbett, A., Preston, P.M., Kirvar, E., Wilkie, G.M., Eckersall, P.D., Hall, R.F. and Brown, C.G.D. 2003. The protozoan parasite, *Theileria annulata*, induces a distinct acute phase protein response in cattle that is associated with pathology. *International Journal for Parasitology*, 33: 1409–1418.

Grewal, A., Ahuja, C.S., Singha, S.P. and Chaudhary, K.C. 2005. Status of lipid peroxidation, some antioxidant enzymes and erythrocytic fragility of crossbred cattle naturally infected with *Theileria annulata*. *Veterinary Research Communications*, 29: 387–394.

Griffiths, M.J., Ndungu, F., Baird, K.L., Muller, D.P., Marsh, K. and Newton, C.R.J.C. 2001. Oxidative stress and erythrocyte damage in Kenyan children with severe *Plasmodium falciparum* malaria. *British Journal of Haematology*, 113: 486–491.

Halliwell, B. and Chirico, S. 1993. Lipid peroxidation: its mechanism, measurement, and significance. *American Journal of Clinical Nutrition* (Suppl. 1), 57: 715S–725S.

Harvey, J.W. 1997. The erythrocyte: physiology, metabolism and biochemical disorders. In: Kaneko, J.J., Harvey, J.W. and Bruss, M.L. (editors), Clinical Biochemistry of Domestic Animals. 5st edition, Academic Press, London, Pp: 182-184.

Hashemi-Fesharaki, R. 1997. Tick-borne diseases of sheep and goats and their related vectors in Iran. *Parasitologia*, 39:115-117.

Jain, N.C. 1993. Essentials of veterinary hematology, 15st (ed.), Lea and Febiger, Philadelphia, Pp: 389–396.

Konukoglu, D., Serin, O., Kemerli, D.G., Serin, E., Hayiroglu, A. and Oner, B. 1998. A study on the carotid artery intima-media thickness and its association with lipid peroxidation. *Clinica Chimica Acta*, 277: 91–98.

Lata, H., Ahuja, G.K., Narang, A.P.S. and Walia , L. 2004. Effect of immobilization stress on lipid peroxidation and lipid profile in rabbits. *Indian Journal of Clinical Biochemistry*, 19: 1–4.

Lykkesfeldt, J. and Svendsen, O. 2007. Oxidants and antioxidants in disease: Oxidative stress in farm animals. *The Veterinary Journal*, 173: 502–511.

May, J.M., Qu, Z.C. and Mendiratta, S.1998. Protection and recycling of alfa-tocopherol in human erythrocytes by intracellular ascorbic acid. *Archives of Biochemistry and Biophysics*, 349: 281–289.

Miller, J.K., Brzezinska-Slebodzinska, E. and Madsen, F.C. 1993. Oxidative stress, antioxidants, and animal function. *Journal of Dairy Science*, 76: 2812–2823.

Murase, T., Ueda, T., Yamato, O., Tajima, M. and Maede, Y. 1996. Oxidative damage and enhanced erythrophagocytosis in canine erythrocytes infected with *Babesia gibsoni*. *Journal of Veterinary Medical Science*, 58: 259–261.

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

Nazifi, S., Razavi, S.M., Mansourian, M., Nikahval, B. and Moghaddam, M. 2008. Studies on correlation among parasitemia and some hemolytic indices in two tropical diseases (theileriosis and anaplasmosis) in Fars province of Iran. *Tropical animal health and production*, 40: 47–53.

Naziroglu, M., Saki, C.E. and Sevgili, M. 1999. The effect of buparvaquone treatment on the levels of some antioxidant vitamins, lipid peroxidation and glutathione peroxidase in cattle with theileriosis. *Journal of Veterinary Medicine B*, 46(4): 233–239.

Paglia, D.E. and Valentine, W.N. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *Journal of Laboratory and Clinical Medicine*, 70: 158–169.

Placer, Z.A., Cushman, L.L. and Johnson, B.C. 1966. Estimation of product of lipid peroxidation (malondialdehyde) in biochemical systems. *Analytical Biochemistry*, 16: 359–364.

Sahoo, A., Patra, R.C., Pathak, N.N. and Dash, P.K. 2001. Enhanced lipid peroxide levels in the erythrocytes of calves with haemoglobinuria. *Veterinary Research Communications*, 25(1): 55–59.

Saleh, M.A. 2009. Erythrocytic oxidative damage in crossbred cattle naturally infected with *Babesia bigemina*. *Research in Veterinary Science*, 86: 43–48.

Saleh, M.A., Al-Salahy, M.B. and Sanousi, S.A. 2009. Oxidative stress in blood of camels (*Camelus dromedaries*) naturally infected with *Trypanosoma evansi*. *Veterinary Parasitology*, 162: 192–199.

Shiono, H., Yagi, Y, Chikayama, Y., Miyazaki, S. and Nakamura, I. 2003. Oxidative damage and phosphatidylserine expression of red blood cells in cattle experimentally infected with *Theileria sergenti*. *Parasitology Research*, 89: 228–234.

Soulsby, E.J.L. 1982. Helminthes, arthropods and protozoa of domesticated animals, 7th (ed.), Bailliere Tindal., London, Pp: 728–739.

Todorova, I., Simeonova, G., Kyuchukova, D., Dinev, D. and Gadjeva, V. 2005. Reference values of oxidative stress parameters (MDA, SOD, CAT) in dogs and cats. *Comparative Clinical Pathology*, 13: 190–194.

Trevisan, M., Browne, R., Ram, M., Muti, P., Freudenheim, J., Carosella, A.N. and Armstrong, D. 2001. Correlates of markers of oxidative status in the general population. *American Journal of Epidemiology*, 154: 348–356.

Wen, J.J., Vyatkina, G. and Garg, N. 2004. Oxidative damage during chagasic cardiomyopathy development: role of mitochondrial oxidant release and inefficient antioxidant defense. *Free Radical Biology and Medicine*, 37: 1821–1833.