Status of lipid peroxidation and antioxidant enzymes in goats naturally infected with *Babesia ovis*

Bijan Esmaeilnejad¹, Mousa Tavassoli¹*, Siamak Asri-Rezaei², Bahram Dalir-Naghadeh² and Hassan Malekinejad³

¹Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran; ²Departments of Clinical Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran; ³Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia, Iran

Abstract

This study aimed to assess lipid peroxidation and antioxidant enzymes in goats naturally infected with *Babesia ovis*. Red blood cell count (RBC), hemoglobin (Hb) concentration, packed cell volume (PCV), malondialdehyde (MDA) concentration, erythrocyte superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) activities and total antioxidant capacity (TAC) were determined in 15 goats naturally infected with *B. ovis* as well as same number of healthy goats. The parasitological diagnosis was confirmed using polymerase chain reaction (PCR) analysis by amplifying a partial 18S rRNA gene sequence of *B. ovis*. Percentage of parasitemia varied from 0.01 to 1%. The activities of erythrocyte GSH-Px, SOD, CAT and TAC were significantly lower (p<0.05) in the infected goats than in healthy ones. MDA concentration in erythrocytes of infected goats was significantly higher in infected goats than in healthy ones (p<0.05). Severity of parasitemia showed a positive correlation with the MDA and negative correlation with PCV, SOD, CAT, GSH-Px and TAC. Also, MDA was negatively correlated with PCV, SOD, CAT, GSH-Px and TAC. The results of this study suggested that oxidative damage to RBCs may contribute to the pathogenesis of anemia in caprine babesiosis.

Keywords

*Babesia ovis*, goats, oxidative stress, antioxidants, anemia

Introduction

Babesiosis is a tick-borne disease of domestic and wild animals caused by species of the genus *Babesia*. The *Babesia* spp. cause fever, anemia, hemoglobinuria and jaundice in small ruminants (Razmi et al. 2003). *Babesia ovis*, *B. motasi* and *B. crassa* were reported from Iran (Uilenberg 2006). *B. ovis* is considered as a highly pathogen organism which caused small ruminants' babesiosis in most part of Iran (Rahbari et al. 2008). The main feature of the disease is hemolytic anemia (Habibi et al. 2004, Sevinc et al. 2007), caused by mechanical damage (Callow and Pepper 1974), autoimmune phenomena (Argon 1976), increase of host erythrocyte permeability (Alkhalil et al. 2007) and erythrophagocytosis by activated macrophage (Saleh 2009).

There is some evidence in support of oxidative stress and lipid peroxidation being a factor in the pathogenesis of anemia in babesiosis. Lipid peroxidation is a general mechanism whereby free radicals induce tissue damage, and is implicated in several diverse pathological conditions (Asri-Rezaei and Dalir-Naghadeh 2006). Lipids especially polyunsaturated fatty acids (PUFA) are sensitive to oxidation, leading to the term lipid peroxidation or the thiobarbituric acid reactive substances (TBARS), of which, malondialdehyde (MDA) is the most abundant (Crnogaj et al. 2010). Determination of MDA allows detection of the degree of lipid peroxidation and the level of free oxygen radicals indirectly (Deger et al. 2009). Ambawat et al. (1999) showed increased oxidative stress and a significant increase in lipid peroxidation in erythrocytes of cattle infected with *Theileria equi*. They concluded that this might be the cause of increased erythrocyte fragility due to membrane lysis.

Oxidative damage to hemoglobin has been shown to cause changes in its structure and function, resulting in denaturation and precipitation of hemoglobin and methemoglobin formation inside erythrocytes. It is markedly increased at the onset of anemia in natural *B. bigemina* infections and an inverse relationship has been observed between methemoglobin levels and the PCV (Saleh 2009). In *B. gibsoni* infections, there is strong evidence of the role of erythrocyte oxidation...
Evaluation of antioxidant status and oxidative stress in goats infected with \textit{B. ovis}  


There are no published reports about erythrocytic oxidative damage and antioxidant defense in caprine babesiosis. Thus, the aim of this study was to determine the activities of catalase (CAT) and erythrocyte glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and total antioxidant capacity (TAC) as important profiles of antioxidant status and the level of malondialdehyde (MDA), as a biomarker of oxidative damage to erythrocytes in goats naturally affected by babesiosis. In addition, the interrelationships of these markers with the degree of parasitemia have been evaluated.

Materials and methods

Source of animals and samples

This study was conducted on 15 goats naturally with occurring cases of caprine babesiosis in the North-West of Iran (West-Azerbaijan Province), where babesiosis due to \textit{B. ovis} is endemic during the summer season (June-September 2009). These goats had a history of tick infestation, anorexia, prolonged listlessness, increased rectal temperature, dyspnea and tachycardia. As a control group, 15 clinically healthy goats reared under the same management and environmental conditions were also sampled. No abnormal clinical signs were observed by physical examination of goats in the control group. Based on laboratory investigations, all the selected goats were also free from other blood parasites.

Blood sampling and parasitological examination

Thin blood smears were prepared of the ear vein from both healthy and infected goats and then stained with Giemsa for microscopic observation of \textit{B. ovis} in erythrocytes. From infected and healthy goats, blood samples were taken from the jugular vein in tubes containing EDTA-K$_2$ for hematological and biochemical analysis. Parasitemia was calculated by counting the number of infected red blood cells (RBCs) in at least 100 examined microscopic fields of vision (Razmi \textit{et al.} 2003). The number of infected cells was then expressed as a percentage. The smears were recorded as negative for \textit{Babesia} spp. if no parasite were observed in 100 oil-immersion fields. Finally, the parasitological diagnosis of \textit{B. ovis} infection was confirmed using polymerase chain reaction (PCR). During sampling, the whole body of each animal was examined for the presence of ticks’ infestation by palpation. The ticks were manually removed, stored in tubes containing 70% ethanol and labelled with collection points noted, and then transferred to the laboratory. Ticks’ species were identified using the standard taxonomic keys (Estrada-Pena \textit{et al.} 2004).

PCR amplification

Not only was PCR performed to detect \textit{B. ovis} in blood of infected animals, but also it used to rule out the presence of \textit{B. ovis} infection in both healthy and infected animals. Extraction of DNA was performed according to the procedure described by Aktas \textit{et al.} (2005) with some modifications. Briefly, 125 µl of the blood sample was added to 250 µl of lysis buffer (0.32 M sucrose, 0.01 M Tris, 0.005 M MgCl$_2$, 1% Triton X-100, pH 7.5) and the mixture was centrifuged at 11600 × g for 1 min. The pellet was washed three times by centrifugation with 250 lysis buffer. The supernatant was discarded and the final pellet was resuspended in 100 µl of PCR buffer [50 mM KCl, 10 mM Tris-HCl (pH 8), 1% Triton X-100, and pH 8.3] containing 50 µg of proteinase K/mI and then incubated at 65°C for 1 h. Finally the sample was boiled at 95°C for 10 min.

A pair of primers, Bbo-F 5’-TGGGCAGGACCTTG- GTTGTTCT-3’ and Bbo-R 5’-CCCGCTAGCGCCGCGC- TAAATA-3’ (GenBank accession numbers AY998123 and AY998124), were used to amplify a 549 bp fragment of the 18S rRNA gene \textit{B. ovis}. The primer’s specificity and sensitivity and also the PCR condition have been described previously by Aktas \textit{et al.} (2005). PCR was carried out in 50 µl total reaction volume containing 5 µl of 10 × PCR buffer, 2 mM MgCl$_2$, 250 µM of each of the four deoxynucleotide triphosphate, 1.25 U Taq DNA polymerase (Fermentas, Germany), 50 pmol of each primer and 5 µl (= 25 ng) of extracted DNA. Amplification of parasite DNA was done in thermocycler CP2-003 (Corbett Research, CP2-003, Australia). Cycling condition for \textit{B. ovis} was 95°C for 5 min, followed by 45 cycles at 94°C for 45 sec, 63°C for 45 sec and 72°C for 1 min with a final extension step of 72°C for 10 min. The PCR products were separated by electrophoresis on 1.5% agarose gel in Tris-Borate-EDTA (TBE) buffer and visualized using ethidium bromide (1 µg/ml) and UV transilluminator (BTS-20 M, Japan).

The positive control for \textit{B. ovis} was kindly provided by professor Rahbari (Faculty of Veterinary Medicine, University of Tehran, Iran). Distilled water used as negative control in PCR amplification.

Hematological examination

Red blood cell (RBC) counts, hemoglobin (Hb) concentration and the packed cell volume (PCV) were determined by an automated hematology analyzer (Autolyser AL 820, Swiss) (Asri-Rezaei and Dalir-Naghadeh 2006). For evaluation of MDA and estimation of antioxidant enzymes, blood samples were centrifuged at 700 × g for 15 min, plasma was separated and packed cells were washed three times with a normal saline solution (0.9%). The washed erythrocytes were then hemolyzed with nine volumes of ice-cold distilled water to prepare 10% RBC hemolysate (Saleh 2009).
Biochemical analysis

Lipid peroxidation in the RBC hemolysate was determined as thiobarbituric acid reactive substance (TBARS) according to Grewal et al. (2005). The method is dependent on forming a color complex between the products of lipid peroxidation and thiobarbituric acid (TBA). Briefly, 0.2 ml of RBC hemolysate were added to 1.3 ml of 0.2 M Tris-KCl buffer of (pH 7.4) and incubated at 37°C for 30 min after which 1.5 ml of TBA were added and the mixture was then heated in a boiling water bath for 10 min. After cooling, 3 ml of pyridine/n-butanol (3:1 v/v) and 1 ml of 1 N NaOH was added to it and mixed by shaking. The absorbance was read at 532 nm against bi-distilled water as a blank. In this assay, 1, 1, 3, 3-tetramethoxypropane was used as a standard. Lipid peroxidation in the RBC hemolysate was expressed as MDA nanomoles per grams of hemoglobin (nmol/g Hb).

The activity of catalase was measured by colorimetric method, described by Asri-Rezaei and Dalir-Naghadeh 2006, that involves two steps. Since the rate of dismutation of hydrogen peroxide to water and oxygen is proportional to the concentration of catalase, samples were first incubated with a known amount of hydrogen peroxide. The remaining hydrogen peroxide, following a fixed incubation period, was then determined by the oxidative coupling reaction of 4-aminophenazone (4-aminoantipyrene, AAP) and 3,5-dichloro-2-hydroxy-benzenesulfonic acid (DHBS) in the presence of \( \text{H}_2\text{O}_2 \) and catalyzed by horseradish peroxide. The resulting quinoneimine dye was measured at 520 nm (Catalase Assay Kit, Oxford Biochemical Research, Inc., USA). Activities of the enzymes were expressed as U/mg Hb.

The activity of GSH-Px, in the RBC hemolysate was measured using the method of Asri-Rezaei and Dalir-Naghadeh (2006); this method is based on the development of a stable yellow color when 2-nitrobenzoic acid is added to sulfhydryl compounds. The amounts of reduced product, thionitrobenzene, were measured by commercially available kits (Ransel test kit, Randox laboratories Ltd. GB) at 412 nm and express as IU/mg Hb.

For evaluation of activity of SOD, superoxide radicals generated by the xanthine oxidase reaction convert 1-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride quantitatively to a formazan dye (Ransel test kit, Randox laboratories Ltd. GB). Conversion of superoxide radicals to hydrogen peroxide by superoxide dismutase inhibits dye formation and serves as a measure of superoxide dismutase activity.

The activity of TAC was determined in the RBC hemolysate according to the methods described by El-Deeb and Younis (2009). In this reaction, ABTS\(^{+\cdot}\) (2, 2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) radicals produced by incubation of ABTS\(^+\) with a peroxidase (metmyoglobin) and \( \text{H}_2\text{O}_2 \). The resulting stable blue-green color was measured at 600 nm (Ransel test kit, Randox laboratories Ltd. GB). Activity of the enzyme was expressed as mmol/l.

Statistical analysis

Statistical analysis of data for hematological parameters and stress oxidative indices were conducted using one way analysis of variance (ANOVA). The relationship between antioxidant enzymes, MDA, percentage of parasitemia and anemia were assessed by using Pearson’s correlation coefficient. Difference were considered significant when \( p<0.05 \). The computer software, SPSS Version 17.0 was used for analysis.

Results

Assessment of parasitemia and molecular confirmation of B. ovis infection

Blood smears prepared from the 15 diseased animals showed the presence of different level of parasitemia ranging from 0.01 to 1% in the RBCs. All of the infected animals were positive by PCR (Fig. 1). On the contrary, there was no amplification of B. ovis DNA from the healthy animals. Clinical examination indicated that infected animals had symptoms such as fever, anorexia and listlessness. In all the infected animals, engorged or semiengorged adult ixodid ticks were found. The ticks found on the infected animals were Rhipicephalus bursa, R. sanguineus and R. turanicus.

Hematological findings

The mean values of the PCV, RBC count and Hb were significantly lower in infected goats compared to the healthy ones (Table I). There was a negative correlation (\( r = -0.881 \),
Table I. Red blood cell count (RBC), hemoglobin (Hb) concentration and packed cell volume (PCV) of RBC in healthy and *B. ovis* infected goats

<table>
<thead>
<tr>
<th>Goats</th>
<th>No. of goats</th>
<th>RBC, $\times 10^{12}$/L</th>
<th>PCV%</th>
<th>Hb g/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>15</td>
<td>6.79 ± 0.098</td>
<td>37.00 ± 0.683</td>
<td>12.33 ± 0.228</td>
</tr>
<tr>
<td>Infected</td>
<td>15</td>
<td>5.58 ± 0.086a</td>
<td>30.00 ± 0.768a</td>
<td>10.00 ± 0.256a</td>
</tr>
<tr>
<td>df</td>
<td>–</td>
<td>1.28</td>
<td>1.28</td>
<td>1.28</td>
</tr>
<tr>
<td>F</td>
<td>–</td>
<td>8.4</td>
<td>56.0</td>
<td>40.7</td>
</tr>
</tbody>
</table>

*aMean differs significantly (p<0.05) in columns.

Table II. Erythrocytic lipid peroxidation (nmol/g Hb), superoxide dismutase (SOD) (IU/mg Hb), glutathione peroxidase (GSH-Px) (IU/mg Hb), catalase (katal/g Hb) activities, total antioxidant capacity (TAC) (mmol/L) and PCV (%) level in healthy and *B. ovis* infected goats

<table>
<thead>
<tr>
<th>Goats</th>
<th>No. of goats</th>
<th>MDA (nmol/g Hb)</th>
<th>SOD (IU/mg Hb)</th>
<th>GSH-Px (IU/mg Hb)</th>
<th>Catalase (katal/g Hb)</th>
<th>TAC (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>15</td>
<td>8.10 ± 0.138</td>
<td>8.01 ± 0.169</td>
<td>74.25 ± 0.676</td>
<td>91.26 ± 0.975</td>
<td>0.47 ± 0.016</td>
</tr>
<tr>
<td>Infected</td>
<td>15</td>
<td>9.33 ± 0.187a</td>
<td>5.86 ± 0.187a</td>
<td>70.75 ± 0.651a</td>
<td>69.96 ± 0.755a</td>
<td>0.36 ± 0.017a</td>
</tr>
<tr>
<td>df</td>
<td>–</td>
<td>1.28</td>
<td>1.28</td>
<td>1.28</td>
<td>1.28</td>
<td>1.28</td>
</tr>
<tr>
<td>F</td>
<td>–</td>
<td>35.68</td>
<td>71.97</td>
<td>33.35</td>
<td>780.0</td>
<td>17.8</td>
</tr>
</tbody>
</table>

*aMean differs significantly (p<0.05) in columns.

Table III. Correlation between oxidative stress, activities of antioxidant enzymes and parasitemia in goats (n = 15) infected with *B. ovis*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PCV</th>
<th>Parasitemia</th>
<th>MDA</th>
<th>GSH-Px</th>
<th>Catalase</th>
<th>SOD</th>
<th>TAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC</td>
<td>1.000</td>
<td>-0.881</td>
<td>-0.766</td>
<td>0.670</td>
<td>0.775</td>
<td>0.874</td>
<td>0.876</td>
</tr>
<tr>
<td>P-value</td>
<td>–</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Parasitemia PC</td>
<td>1.000</td>
<td>0.788</td>
<td>-0.902</td>
<td>-0.588</td>
<td>-0.902</td>
<td>-0.800</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>–</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td>0.001</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>MDA PC</td>
<td>1.000</td>
<td>-0.674</td>
<td>-0.639</td>
<td>-0.758</td>
<td>-0.829</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>–</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td></td>
</tr>
<tr>
<td>GSH-Px PC</td>
<td>1.000</td>
<td>0.280</td>
<td>0.759</td>
<td>0.502</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>–</td>
<td>0.135</td>
<td>&lt;0.0005</td>
<td>0.005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catalase PC</td>
<td>1.000</td>
<td>0.766</td>
<td>0.886</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>–</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD PC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.000</td>
<td>0.808</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAC PC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0005</td>
<td></td>
</tr>
</tbody>
</table>

*aPearson correlation.
p<0.0005) between the intensity of parasitemia rate and PCV (Table III).

**Evaluation of lipid peroxidation and antioxidant enzymes activity**

The summary statistics of the measured parameters in healthy and infected goats are presented in Table II. MDA evaluation in erythrocytes indicated that goats with *B. ovis* infection had significantly (p<0.05) higher lipid peroxide levels than the healthy goats. In addition, corpuscular MDA concentration was positively correlated with percentage parasitemia (r = 0.788, p<0.0005) and negatively correlated with PCV (r = -0.76, p<0.0005) (Table III). It seems that, *B. ovis* stimulates oxidative damage and induces MDA production.

Significant differences (p<0.05) in activities of erythrocyte GSH-Px and SOD between healthy goats and those infected with *B. ovis* were evident (Table II). There was a significant inverse relationship between percentage parasitemia and the activities of erythrocyte GSH-Px (r = -0.58, p<0.0005) and SOD (r = -0.9, p<0.0005). There was also an inverse relationship between the activities of these antioxidant enzymes and MDA levels (r = -0.63, p<0.0005; r = -0.67, p<0.0005, respectively, for erythrocyte GSH-Px and SOD, Table III). As Table II shows, a significant difference (p<0.05) was evident in the activity of erythrocyte catalase between healthy and infected goats. In addition, the severity of parasitemia was negatively correlated with catalase activity (r = -0.588, p<0.05).

With progression of parasitemia severity, there was a significant reduction (r = -0.8, p<0.05) in the level of erythrocyte total antioxidant capacity (Table III). Total antioxidant capacity and catalase was negatively correlated with percentage of parasitemia (r = -0.8, p<0.0005), (r = -0.9, p<0.0005) and MDA concentration (r = -0.82, p<0.0005), (r = -0.75, p<0.0005), respectively (Table III).

**Discussion**

The aim of this study was to evaluate the cytochemical alteration of RBCs in anemic goats suffering from caprine babesiosis. Our findings showed that oxidative damage to RBCs may be involved in pathogenesis and onset of anemia in babesiosis caused by *B. ovis*.

The anemia following Babesia infection was attributed to mechanical damage, autoimmune phenomena and erythrophagocytosis. The anemia observed in the present study is consistent with those reported earlier (Varshney et al. 2003, Bicek et al. 2005, Chaudhuri et al. 2008, Saleh 2009). In addition, the negative correlation between percentage of parasitemia and PCV values in the current study may be due to destruction of *B. ovis*-infected erythrocytes. Similar findings have been reported by previous studies (Ambawat et al. 1999, Bicek et al. 2005, Crnogaj et al. 2010).

Lipid peroxidation is an ongoing physiological process, but there are some evidence that lipid peroxidation incorporate in pathogenesis of several parasitic diseases (Kiral et al. 2005). The erythrocytes membrane is rich in polyunsaturated fatty acids (PUFA), a primary target for reactions involving free radicals, and very susceptible to lipid peroxidation (Sahoo et al. 2001, Kumar et al. 2006, Saleh 2009). No report is available about erythrocytic oxidative changes in *B. ovis*-infected goats; however, in accordance with the findings of other studies (Commins et al. 1988, Chaudhuri et al. 2008, Deger et al. 2009, Crnogaj et al. 2010), our results indicated that the lipid peroxidation in erythrocytes of affected goats increased MDA production.

In bovine babesiosis caused by *B. bovis*, infection stimulates the production of interleukin-1β, interleukin-12, gamma interferon (IFN-γ) and tumor necrosis factor-α (TNF-α). These mediators activate mononuclear phagocytes/macrophages to release reactive nitrogen intermediates (Shoda et al. 2000). Stich et al. (1998) showed that *B. bovis*-infected erythrocytes stimulate inducible nitric oxide synthesis (iNOS) transcription and nitric oxide production by the activated macrophages of cattle. Also, increased nitric oxide production during equine babesiosis caused by *B. caballi* and *T. equi* has been previously reported (Deger et al. 2009). Therefore, the increased production of these free radicals leads to augmented oxidative stress to macromolecules and biomembranes, resulting in enhanced lipid peroxidation and MDA production (Saleh 2009). The high concentration of lipid peroxides from dogs with babesiosis can probably be ascribed to multiplications of *B. gibsoni* (Otsuko et al. 2001, Kumar et al. 2006). However, high level of MDA concentration in *B. canis*-infected dogs could be related to enhanced lipid peroxidation and also because of decreased kidney function (Crnogaj et al. 2010).

In *B. ovis* infection the severity of disease is determined by the degree of parasitemia (Sevinc et al. 2007). In the current study, the positive correlation between percentage of parasitemia and concentration of erythrocytic MDA suggest that the severity of disease is directly related to erythrocyte lipid peroxidation. In *B. bovis* infection, Court et al. (2001) observed that around the time of peak parasitemia, peripheral bovine monocytes and neutrophils engage in enhanced oxidative burst and production of oxidative radicals. The negative correlation between erythrocyte MDA and PCV values suggesting that the increase in lipid peroxidation may be one of the factors involved in the reduction of PCV values and accordingly occurrence of anemia. Shiono et al. (2003), Asiri-Rezaei and Dalir-Naghadeh (2006), Saleh (2009) found a marked increase of erythrocytic MDA levels in proportion to the decrease in PCV and the increase in parasitemia in cattle infected with *T. sergenti*, *T. annulata* and *B. bigemina*, respectively.

In this study, with increase in parasitemia severity in infected goats, a significant decrease (p<0.05) was observed in the activity of GSH-Px, which is in accordance with the find-
ings of Bicek et al. (2005) and Deger et al. (2009) in ovine and equine piroplasmosis, respectively. GSH-Px activity is a major mechanism for intracellular decomposition of lipid peroxidase (Grewal et al. 2005). Hafeman and colleagues (1974) also proposed that GSH-Px plays a crucial role in preventing membranes from peroxide damage induced by lipid peroxides. This reaction is important because the accumulation of H₂O₂ might decrease the lifespan of erythrocytes by increasing the rate of oxidation of hemoglobin to methemoglobin (Grewal et al. 2005). Reduction of GSH-Px activity could be due to azotemia from injuries to the kidneys (Buranakarl et al. 2009). Evaluation of erythrocytic SOD activity in B. ovis-infected goats showed that with increasing parasitemia and oxidative damage in parasitemized erythrocytes (increased MDA concentration), the activity of this enzyme significantly reduced.

There appear to be no reports on the alteration of SOD activity in B. ovis infection to compare with the results in the present study. However, low activity of SOD has been reported in T. annulata-infected cattle and buffaloes (Asri-Rezaei and Dalir-Naghadeh 2006, El-Deeb and Younis 2009, respectively) and as well as in Plasmodium vivax-infected erythrocytes (Erel et al. 1997). It appears that, during piroplasmosis, as with GSH-Px, SOD plays an important role in protection of erythrocytes against oxidative damage. However, in contrast to our results, Chaudhuri et al. (2008) reported a significant rise in the activity of SOD in infected dogs with B. gibsoni. Although, the mechanism of increased activity of SOD in infected erythrocytes cannot be explained explicitly, it is likely that up-regulation of the synthesis of SOD is driven by the body’s homeostatic mechanisms to counter oxidative damage due to parasitemia and multiplication of parasites inside the cells (Chiwakata et al. 2000, Chaudhuri et al. 2008). It may be associated with a high percentage of reticulocytes in the infected dogs because the activity of enzymes is higher in reticulocytes compared with mature erythrocytes (Yamasaki et al. 2000, Otsuko et al. 2001).

Evaluation of catalase activity in the infected goats showed that by increasing the severity of parasitemia, activity of this enzyme was reduced significantly. It has been reported that catalase and GSH-Px are equally important in the defense of human erythrocytes against generating reactions (Harvey 1989). However, the results of this study indicated that catalase may be acting collaboratively with GSH-Px to scavenge H₂O₂ for the protection of erythrocytes infected by Babesia. Our results agree with the finding of El-Deeb and Younis (2009). However, in contrast with our results, Chaudhuri et al. (2008) reported a significant rise in catalase activity in infected dogs with B. gibsoni. This observed elevation of catalase activity could also be attributed to a high percentage of reticulocytes. A reduction in the level of TAC in infected goats may probably be ascribed to the consumption of antioxidant enzymes as free radical scavengers during the oxidative process in natural B. ovis infections in goats. In the current study, the negative correlation of antioxidant status with MDA concentration and parasitemia rate indicate the existence of a link between the enhanced erythrocytic oxidation and reduction of these antioxidant indices, and suggests that enhanced oxidation of the erythrocytes may not only be due to increased free radical generation but also could be exacerbated by the inefficient antioxidant capacity that results in erythrocytic destruction and progression of anemia in B. ovis infection in goats.

In conclusion, the results of the present study showed significant increase in lipid peroxidation of the membrane of erythrocytes of goats suffering from babesiosis. The level of the antioxidant enzymes in the erythrocytes of affected goats decreased as severity of the anemia and parasitemia increased. It seems that antioxidant mechanisms of erythrocytes that protect them against oxidative damage may be distributed by Babesia infection.

Acknowledgements. This project was funded by Urmia University. We would like to thank Dr. Jamal Akhari for his kind help during sample collection. We also thank Mr. Armen Badali and Mr. Hamed Tabatabaei for their technical support.

References
Chiwakata C.B., Hemmer Ch.J. Dietrich M. 2000. High level of nitric oxide synthase mRNA are associated with increased monocyte count in blood and have a beneficial role in Plasmodium falciparum malaria. *Infection and Immunity*, 68, 394–399. DOI: 0019-9567/00/$04.00+0.


