Malondialdehyde levels in serum of dogs infected with *Babesia canis*

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ABSTRACT: Babesiosis is a common tick born disease of dogs in tropical and subtropical regions of the world caused by different species of *Babesia*. The aim of the present study was to confirm the presence of oxidative stress by examining serum malondialdehyde (MDA), an end product of lipid peroxidation, in 35 dogs naturally infected with *Babesia canis (B. canis)*. MDA was examined in 14 healthy dogs as well. Blood smears were prepared from peripheral blood and they showed the presence of *B. canis* in infected dogs. *B. canis* was confirmed using the PCR (Polymerase chain reaction) method. On the basis of clinical and laboratory data the 35 infected dogs were clinically classified into two groups, complicated (seven dogs) and uncomplicated (28 dogs). The noted complications were renal dysfunction (5/7), hepatic dysfunction (3/7), muscular involvement (2/7) and ARDS (1/7). Levels of blood urea nitrogen concentration (BUN), creatinin, total bilirubin, alanin aminotranspherase (ALT), alkaline phosphatase (AP), and gammaglutamil aminotranspherase (GGT) were significantly increased in dogs with complicated versus uncomplicated babesiosis. Furthermore the uncomplicated group of dogs was, depending on the severity of anaemia, classified as suffering from severe, moderate or mild disease. Levels of serum MDA were significantly higher in sick dogs (36.90 μmol/l ± 13.95) than healthy animals (8.13 μmol/l ± 1.78). There was no significant statistical difference in serum MDA levels between dogs with complicated (38.48 μmol/l ± 12.11) and uncomplicated babesiosis (36.50 μmol/l ± 14.55). Comparison of the groups based on the severity of anaemia showed that there was no significant statistical difference in serum MDA levels between them. The study demonstrated the involvement of oxidative damage in dogs naturally infected with *B. canis*.

Keywords: babesiosis; dog; oxidative stress; malondialdehyde (MDA)

Canine babesiosis is a common tick borne disease of worldwide importance. In Europe it is caused by species of the *Babesia* genus: *Babesia canis (B. canis)*, *Babesia gibsoni (B. gibsoni)* and *Babesia microti*-like piroplasms (Uilenberg et al., 1989; Taboada and Merchant, 1991; Camacho et al., 2001). Previous studies have suggested that *B. canis* should be divided into three biologically and immunologically distinct subspecies: *B. canis canis*, *B. canis vogeli* and *B. canis rossi* (Uilenberg et al., 1989). However, additional molecular studies have shown that these three groups of parasites do not cluster in a single clade, which suggests that they are not subspecies (Carret et al., 1999) so the classical nomenclature (*B. canis*, *B. rossi* and *B. vogeli*) will be used here as suggested by Schetters at al. (1997b), Carret et al. (1999), Passos et al. (2005), Schetters (2005) and Uilenberg (2006). Canine babesiosis caused by *B. canis* is a very common cause of morbidity and mortality in dogs in Croatia, especially in the area of the capital city Zagreb (Caccio et al., 2002; Matijatko et al., 2007; Beck et al., 2009; Brkljacic et al., 2010). The disease can be clinically classified into uncomplicated and complicated forms. Uncomplicated babesiosis has been suggested to be a consequence of haemolysis (Jacobson and

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Clark, 1994) while complicated canine babesiosis has been suggested to be a consequence of the development of systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS), both of which are cytokine-mediated phenomena (Jacobson and Clark, 1994; Welzl et al., 2001). However, recent publications showed that both uncomplicated and complicated babesiosis appear to be the result of host inflammatory responses (Matijatko et al., 2007; Schetters et al., 2009). The major mediators of this response are cytokines, nitric oxide, free oxygen radicals, eicosanoids, and platelet-activating factor (Purvis and Kirby, 1994).

Clinical signs of uncomplicated babesiosis include pale mucous membranes, fever, anorexia, depression, splenomegaly and water hammer pulse (Taboada and Merchant, 1991). Clinical manifestations of the complicated form of babesiosis depend on the particular complications that develop. The most commonly reported complications of canine babesiosis are acute renal failure, cerebral babesiosis, coagulopathy, icterus and hepatopathy, immune-mediated haemolytic anaemia (IMHA), acute respiratory distress syndrome (ARDS), haemoconcentration, and shock (Lobetti, 1998).

The quantity of the destroyed erythrocytes is usually much higher than the degree of parasitaemia, suggesting that non-parasited erythrocytes may also be damaged (Murase and Maede, 1990). In experimental *B. rossi* infection, there was a marked decrease in haematocrit long before parasites were detectible in peripheral blood (Shetters et al., 1997a). This early change was hypothesized to be caused by hemodilution, splenomegaly and sequestration in the spleen (Maegraith et al., 1957; Schetters et al., 1997a, 1998, 2009). This mechanism may include autoimmune haemolysis (Reyers et al., 1998), reduced red cell deformability (Dondrop et al., 1999) and increased oxidative damage (Murase et al., 1995; Morita et al., 1996; Otsuka et al., 2001; Rezaei and Dalir-Naghadeh, 2006). A possible role of the highly reactive oxygen free radicals in the pathogenesis of parasitic infections has been an active area of research over the past ten years (Biswas et al., 1997; Erel et al., 1997; Oliveira and Cechini, 2002). The main targets of free oxygen radical activity are polyunsaturated fatty acids (PUFAs) in membrane phospholipids, the modification of which result in disorganization of the cellular framework and function (Patterson and Leacke, 1998).

Under oxidative stress PUFAs are highly susceptible to lipid peroxidation (Solans et al., 2000). Lipid peroxidation is a well-established mechanism of cellular injury and is used as an indicator of oxidative stress in cells and tissues (Magni et al., 1994). Malondialdehyde (MDA), an end product of polyunsaturated fatty acid oxygenation, is a reliable and commonly used biomarker for assessing lipid peroxidation (Moore and Roberts, 1998).

In this present study, we investigated MDA as an indicator of lipid peroxidation, and its possible role in the pathogenesis of babesiosis in dogs naturally infected with *B. canis.*

MATERIAL AND METHODS

Animals. The study was performed on two groups of animals. Thirty five dogs naturally infected with *B. canis* were included in Group 1. Dogs in this group were aged between three months and 10 years, and were of various breeds and gender. All the dogs infected by *B. canis* were presented at the Clinic for Internal Diseases, Faculty of Veterinary Medicine, University of Zagreb, Croatia, with clinical signs of acute babesiosis (including fever, lethargy, ticks found by the owner or veterinarian, pale mucous membranes, anaemia, jaundice, haemoglobinuria or haematuria, anorexia, splenomegaly, tachycardia and vomiting). The diagnosis was confirmed by microscopical examination of Romanowsky-stained peripheral blood smears, with findings of large pyriform parasites within the infected erythrocytes. All of the infected thirty five dogs were included in a bigger study conducted by Beck et al. (2009), in which the babesia were characterized by the Polymerase chain reaction method (PCR). All the dogs included in this study were infected by *B. canis*. A single dose (6 mg/kg of body weight) of imidocarb dipropionate (Imizol® 12%, Schering-Plough) was administered to all the dogs with confirmed babesiosis, subcutaneously on the day of admission. On the basis of clinical manifestations and laboratory data the affected dogs were divided into two groups: complicated (Group 1.1.) and uncomplicated (Group 1.2.) babesiosis (Jacobson and Clark, 1994). An animal was classified as complicated if one of the following criteria were fulfilled: renal dysfunction (serum creatinine concentration of more than 180 μmol/l), hepatic dysfunction (both alanine aminotransferase (ALT) greater than 176 IU/l and alkaline phosphatase (AP)

greater than 360 IU/l), respiratory system dysfunction (radiographic evidence of pulmonary oedema or dyspnoea), and muscular involvement (creatine phosphokinase (CPK) more than 600 IU/l). We included a bilirubin serum level greater than 100 μmol/l as an additional criterion for hepatic dysfunction (Weiser, 1992), and an animal with a bilirubin level greater than 100 μmol/l was classified as having hepatic dysfunction even if ALT and AP were within reference range (Matijatko et al. 2009). Animals that fulfilled two or more organ dysfunction criteria were classified as having MODS.

Furthermore, depending on the severity of anaemia, dogs with uncomplicated babesiosis were classified as suffering from severe (haematocrit (HCT) < 16; Group 1.2.1.), moderate (16 ≤ HCT < 35; Group 1.2.2.), or mild disease (HCT ≥ 35; Group 1.2.3.) (Jacobson and Clark, 1994). Group 2 consisted of 14 clinically healthy mongrel dogs of both sexes, and with a similar age distribution to the infected dogs. The dogs were deemed healthy on the basis of clinical and laboratory data.

Blood analyses. The blood samples were collected from the cephalic vein on the day of admission to the Clinic in 2007. The samples were placed in tubes with EDTA for haematological analysis and tubes with no anticoagulant which were centrifuged at 1200 × g. A portion of the obtained serum was used for establishing biochemical profiles while the remainder was stored at –70°C until it was used for analyzing MDA. Complete blood count was analyzed using an automatic haematology analyzer (System 9120; Serono Baker Diagnostic). Biochemical profiles were generated according to standard methods using an automated biochemistry analyzer (Olympus AU 600, Olympus Diagnostica GMBH). The biochemistry panel included the following parameters: blood urea nitrogen (BUN), creatinine, total protein (TP), albumin, ALT, AP, γ-glutamyl transferase (GGT), blood glucose, total bilirubin and CPK.

Lipid peroxidation was assayed by measuring the malondialdehyde (MDA) concentration in the serum as thiobarbituric acid (TBA) – reactive material. MDA is formed as a secondary product when TBA and polyunsaturared fatty acid are heated in an acidic medium, its absorbance being measured at 523 nm (Uchiyama and Mihara, 1997).

PCR. DNA was extracted from 200 μl of EDTA-anti-coagulated whole venous blood from each dog, using a DNA blood and tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The PCR was performed as previously described (Beck et al., 2009).

Statistical analysis. The data was analyzed using the Tukey HSD test and a *P*-value of < 0.05 was considered statistically significant.

RESULTS

Thirty five cases fulfilled the selection criteria for acute canine babesiosis and were included in the study. On the day of admission, all of the dogs included in this study presented with one or more of the following clinical signs: depression (34/35), anorexia (29/35), pale mucous membranes (26/35), icteric mucous membranes (8/35), fever (20/35), hypothermia (2/35), shock (2/35), splenomegaly (11/35) and water hammer pulse (4/35). The haematological and biochemical parameters are shown in Tables 1, 2, and 3.

Red blood cell count (RBC), HMT and platelet count (PLT) were significantly different ($P < 0.0005$) in dogs with babesiosis in comparison to the control group. PLT count was significantly different ($P < 0.05$) in dogs with complicated versus uncomplicated babesiosis (Table 1).

Table 1. Haematology parameters in healthy dogs and dogs infected with *Babesia canis*

Haematology parameters	Healthy dogs	Infected animals	Complicated babesiosis	Uncomplicated babesiosis
RBC ($\times 10^{12}$/l)	7 ± 0.31	5 ± 1.29*	5 ± 0.98	5 ± 1.37
HCT (%)	53 ± 2.47	37 ± 9.05*	37 ± 7.34	37 ± 9.55
WBC ($\times 10^{9}$/l)	10 ± 1.57	7 ± 4.13	9 ± 7.27	7 ± 2.93
PLT ($\times 10^{9}$/l)	258 ± 59.59	30 ± 30.05*	65 ± 48.08**	22 ± 17.89

RBC = red blood cell count; HCT = haematocrit; WBC = white blood cell count; PLT = platelet count
*the infected group is significantly different than control one ($P < 0.0005$)
**the complicated babesiosis group is significantly different than the uncomplicated babesiosis group ($P < 0.05$)

Table 2. Biochemistry parameters in healthy dogs and dogs infected with *Babesia canis*

Biocemistry parameters	Healthy dogs	Infected animals
BUN (mmol/l)	5 ± 1.48	12 ± 15.05
Creatinine (μmol/l)	85 ± 19.2	133 ± 145.82
TP (g/l)	67 ± 2.65	59 ± 9.05*
Albumin (g/l)	34 ± 2.5	27 ± 4.77***
Total bilirubin (μmol/l)	4 ± 1.63	44 ± 115.45
Blood glucose (mmol/l)	5 ± 0.47	6 ± 1.09*
ALT(IU/l)	36 ± 7.99	161 ± 464.51
AP (IU/l)	43 ± 32.53	161 ± 114.59**
GGT (IU/l)	2 ± 0.67	3 ± 1.98
CPK (IU/l)	117 ± 47.37	261 ± 289.32

BUN = blood urea nitrogen concentration; TP = total protein concentration; ALT = alanin aminotranspherase; AP = alkaline phosphatase; GGT = gammaglutamil aminotranspherase; CPK = creatinine phosphokinase
*the infected group is significantly different than control one ($P < 0.05$)
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TP ($P < 0.05$), albumin ($P < 0.0005$), blood glucose ($P < 0.05$) and AP ($P < 0.005$) were significantly different in dogs with babesiosis in comparison to the control group (Table 2).

BUN ($P < 0.0005$), creatinin ($P < 0.005$), total bilirubin ($P < 0.005$), ALT ($P < 0.05$), AP ($P < 0.05$) and GGT ($P < 0.05$) were significantly different in dogs with complicated versus uncomplicated babesiosis (Table 3).

Uncomplicated babesiosis was established in 28 out of 35 dogs and complicated babesiosis was determined in the remaining seven dogs. The noted complications were renal dysfunction (5/7), hepatic dysfunction (3/7), muscular involvement (2/7) and ARDS (1/7). Two of the animals had MODS, one had two-organ dysfunction (kidney and liver) and one had four-organ dysfunction (kidney, liver, lungs and muscles). According to the severity of anaemia

Table 3. Biochemistry parameters in dogs with complicated and uncomplicated babesiosis

Biocemistry parameters	Complicated babesiosis	Uncomplicated babesiosis
BUN (mmol/l)	33 ± 24.32***	7 ± 2.87
Creatinine (μmol/l)	317 ± 259.57**	87 ± 28.96
TP (g/l)	63 ± 11.44	58 ± 8.36
Albumin (g/l)	27 ± 6.48	27 ± 4.4
Total bilirubin (μmol/l)	177 ± 222.76**	11 ± 6.58
Blood glucose (mmol/l)	5 ± 2.06	6 ± 0.65
ALT (IU/l)	564 ± 1023.52*	65 ± 36.0
AP (IU/l)	290 ± 195.24*	131 ± 60.82
GGT (IU/l)	5 ± 3.31*	3 ± 1.28
CPK (IU/l)	369 ± 621.45	237 ± 152.14

BUN = blood urea nitrogen concentration; TP = total protein concentration; ALT = alanin aminotranspherase; AP = alkaline phosphatase; GGT = gammaglutamil aminotranspherase; CPK = creatinine phosphokinase
*the complicated babesiosis group is significantly different than the uncomplicated babesiosis group ($P < 0.05$)
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Table 4. Concentration of MDA in healthy dogs and dogs infected with *Babesia canis*

Group (No.)	Characteristic	MDA (μmol/l)		
		Minimum	Maximum	Mean ± SD
1 (n = 35)	all infected animals	17.06	75.02	36.90 ± 13.95*
1.1. (n = 7)	complicated case of babesiosis	19.24	51.01	38.48 ± 12.11*
1.2. (n = 28)	uncomplicated case of babesiosis	17.06	75.02	36.50 ± 14.55*
1.2.1.(n = 1)**	HCT < 16	×	×	×
1.2.2. (n = 9)	HCT 16 ≤ HCT < 35	25.50	58.15	38.93 ± 13.04*
1.2.3.(n = 18)	HCT ≥ 35	17.06	75.02	34.93 ± 15.75*
2 (n = 14)	healthy dogs	5.36	11.37	8.13 ± 1.78

HCT = haematocrit; MDA = malondialdehyde
*the infected group is significantly different than control one (P < 0.0005)
**group 1.2.1. has only one case and therefore cannot be statistically analyzed. MDA is 42.98 μM

uncomplicated babesiosis was severe in 1 out of 28 dogs, moderate in 9 out of 28 dogs, and mild in 18 out of 28 dogs.

Twenty eight dogs with uncomplicated babesiosis showed improvement in their clinical symptoms within twenty-four hours after the anti-babesial treatment. Seven complicated cases were additionally treated with intravenous fluids (colloids and crystalloids), oxygen supplementation via intranasal tubes or oxygen cage and intravenous antibiotics. The treatment was successful in three cases and four dogs died despite the treatment.

There was a significant increase in serum MDA levels (P < 0.0005) between the healthy control group (8.13 μmol/l ± 1.78) and dogs with diagnosed babesiosis (36.90 μmol/l ± 13.95; Table 4) but there was no significant statistical difference in serum MDA levels between dogs with complicated (38.48 μmol/l ± 12.11) and uncomplicated babesiosis (36.50 μmol/l ± 14.55; Table 4). The concentration of MDA in the serum of uncomplicated babesiosis sufferers showed no significant statistical difference in regard to the degree of anaemia (Table 4).

DISCUSSION

Canine babesiosis is an important disease of worldwide significance whose effects can range from relatively mild to fatal. In Croatia canine babesiosis occurs predominantly in its uncomplicated form (Matijatko et al., 2007, 2009; Baric Rafaj et al., 2009). The complicated form of babesiosis occurs seldom, but the outcome is often poor. In this study the complicated form was present in 20 percent of all cases. The mortality rate in all the studied dogs was 11.4 percent which is similar to other mortality rates recorded in studies of canine babesiosis in Croatia (Matijatko et al., 2009) as well as South African studies of canine babesiosis caused by *B. rossi* (Collet, 2000; Nel et al., 2004). Contrary to these findings, in a study of canine babesiosis in Poland conducted by Adaszek et al. (2009), the mortality rate was much lower (3.9 percent). The explanation for this could be the presence of different strains of *B. canis* in Croatia. Different strains of *B. canis* and *B. rossi* have been identified and been proven to cause different clinical forms of babesiosis (Carcy et al., 2006; Matjila et al., 2009). Moreover, different babesia strains can be directly linked with the outcome and prognosis of babesiosis (Matjila et al., 2009). In the group of dogs with complicated babesiosis the mortality rate was 57.1 percent. The most common complication was renal dysfunction which is similar to the results of other studies on *B. canis* (Adaszek et al., 2009; Matijatko et al., 2009).

Highly reactive oxygen free radicals have a role in the pathogenesis of various parasitic infections including *Babesia*, *Leishmania*, *Hepatozoon*, *Ehrlichia*, *Theileria* and *Plasmodium* parasites (Murase et al., 1995; Bildik et al., 2004; Kiral et al., 2005; Kumar et al., 2006; Rezaei and Dalir-Naghadeh, 2006). However, to the best of our knowledge, levels of serum MDA have not been previously reported in dogs infected with *B. canis*. MDA is excreted in urine, blood, and other body fluids and therefore serves as a marker of lipid peroxidation and the presence of oxidative stress (Marks et al., 1996).

The results of the current study demonstrate that there is a significant increase in concentrations of serum MDA ($P < 0.0005$) in dogs with diagnosed babesiosis versus the healthy control group. Increased levels of MDA have been reported in *B. gibsoni* infection (Murase et al., 1995; Otsuka et al., 2001; Chaudhuri et al., 2008) and in a mixed infection of *Erlichia canis* and *B. gibsoni* (Kumar et al., 2006).

Anaemia in babesiosis has been investigated in numerous studies. Several mechanisms that can cause anaemia have been proposed, such as sequestration in the spleen, autoimmune haemolysis, reduced red cell deformability, haemodilution and increased oxidative damage (Maegraith et al., 1957; Murase et al., 1995; Schetters et al., 1997a, 1998, 2009; Reyers et al., 1998; Dondrop et al., 1999). Haemodilution as a cause of early anaemia was suggested by Schetters et al. (2009). In their study of experimental babesiosis evidence of haemodilution was provided by decreased haematocrit and plasma creatinine values with their linear correlation. In our study creatinin values were not significantly different in the canine babesiosis group compared with healthy dogs but the creatinin value was significantly higher ($P < 0.005$) in the group of dogs with complicated babesiosis compared to uncomplicated babesiosis. This difference in results could be explained by the fact that this was a clinical study and the investigated group consisted of dogs that were of variable breed, sex and age. Moreover, we could not be certain when exactly infection occurred. It is possible that we missed the early phase of infection in which the fall in creatinine value occurred. Another explanation for the higher values of creatinine in our study compared to the study conducted by Schetters et al. (2009) could be the presence of kidney failure in the group of complicated babesiosis accompanied with increased creatinine values. Although in our study the creatinine values did not support the haemodilution hypothesis, the significantly lower concentrations of total plasma proteins ($P < 0.05$) and albumin ($P < 0.0005$) recorded in infected dogs compared to healthy dogs support the occurrence of haemodilution in canine babesiosis. There are indications that oxidative stress and lipid peroxidation play a role in the pathogenesis of anaemia in some protozoan diseases (Rezaei and Dalir-Naghadeh, 2006). Intensive lipid peroxidation in biological membranes causes disturbances of its structural integrity, loss of fluidity, decrease in membrane potential, and increased permeability to ions (Gutteridge, 1995). These changes lead to rupture of the membrane and release of cell contents (Halliwell and Chirico, 1993). Mata (1990) opined that due to lipid peroxidation there is an accumulation of oxidative ions in red blood cells which causes their lysis. Of interest in this study was also the relationship between increased MDA production and the severity of anaemia in dogs with uncomplicated babesiosis. The concentration of MDA in the serum of uncomplicated babesiosis showed no significant statistical difference with respect to the degree of anaemia. Our finding is contrary to the report involving cattle infected with *Theileria sergenti* and *Theileria annulata* where MDA levels began to increase remarkably in proportion to the degree of anaemia (Shiono et al., 2003; Rezaei and Dalir-Naghadeh, 2006). The reason for that could also be the insufficient number of dogs for each category of anaemia in this study.

Many parasites including protozoa are sensitive to oxidative stress. Sensitivity to oxidative stress has been reported in malaria (Rockett et al., 1991), hepatozoonosis (Kiral et al., 2005), tropical theileriosis (Visser et al., 1995) and babesiosis (Stich et al., 1998). Reactive oxygen species (ROS) and Reactive Nitrogen Species (RNS) are powerful oxidants and nitrating species that can inactivate enzymes and initiate the process of lipid peroxidation and nitration, which leads to radical chain reactions that further damage membranes, nucleic acids and proteins (Muller et al., 2003). These processes may ultimately lead to the killing of parasitic organisms (Rockett et al., 1991; Kiral et al., 2005). As a defense mechanism parasites possess cellular chemicals and antioxidant enzymes that directly neutralize ROS and RNS. During recent years, several antioxidant and detoxification systems of parasitic protozoa have been identified and their role in the survival of the parasites investigated (Muller et al., 2003). These so-called "redox proteins" such as thioredoxin reductase (Kanzok et al., 2000), glutamate dehydrogenase (Wagner et al., 1998) and glutathione S-transferase (Harwaldt et al., 2002) from *Plasmodium falciparum*, trypanothione synthetase from *Trypanosoma cruzi* (Schmidt and Krauth-Siegel, 2002) and 3-mercaptopyruvate sulfurtransferase from *Leishmania major* (Williams et al., 2003) have been shown to be essential for the survival of protozoan parasites.

On the other hand, oxidative stress could negatively affect organ function and thus overall survival (Crimi et al., 2006). Serum malondialdehyde

level was found to be significantly elevated in falciparum malaria-induced acute renal failure cases when compared with uncomplicated falciparum malaria (Nanda et al., 2004). Contrary to these findings, in our study, there was no significant statistical difference in serum MDA levels between dogs with complicated versus uncomplicated babesiosis. MDA is present in serum and excreted in urine. The higher levels of MDA in the serum could be due to enhanced lipid peroxidation, but also because of decreased kidney function. The creatinine levels were significantly higher in the group of dogs with complicated babesiosis which was in concordance with the clinical findings of kidney failure only in the complicated babesiosis group. Since the results of our study showed no significant difference in MDA levels between dogs with uncomplicated and complicated babesiosis, the higher levels of MDA in serum could not be explained with decreased glomerular filtration and therefore could be attributed to enhanced lipid peroxidation.

The products of oxidative stress may contribute to protective immune responses against the parasite if produced in optimal amounts (Kiral et al., 2005; Visser et al., 1995), but can also contribute to pathogenesis when produced in excessive amounts Visser et al., 1995). Therefore, the key determinant for favourable outcome in babesiosis may be the balance between oxidant and antioxidant processes.

In conclusion, the results of the present study showed that oxidative damage, as evidenced by MDA serum levels in dogs naturally infected with *B. canis*, may play an important role in the pathophysiology of babesiosis. The present results should be strengthened by further investigations on larger number of dogs into the exact role of oxidative stress in uncomplicated and, particularly complicated babesiosis.

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