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The Role of Oxidative Stress in the Pathogenesis of Babesiosis in Dogs

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Abstract: *Babesia bacteria invade red blood cells, causing cell destruction and a variety of other systemic issues in dogs. This is the source of the canine sickness known as babesiosis. In this work, the association between oxidative stress and canine babesiosis is investigated by detecting levels of malondialdehyde (MDA), a biomarker of lipid peroxidation and oxidative damage. The study compared ten dogs diagnosed with babesiosis with ten healthy control dogs. Infected dogs were found to have significantly higher MDA levels ($p<0.01$). These results highlight the key role of oxidative stress in the development of canine babesiosis and suggest that antioxidant therapy may be beneficial in the treatment of canine babesiosis. Further research into oxidative stress as a potential target for intervention in problematic dog populations is warranted.*

I. INTRODUCTION

The biochemical imbalance known as oxidative stress is characterized by increased production of reactive oxygen species (ROS) and decreased antioxidant defenses. ROS, such as superoxide anions and hydrogen peroxide, are highly sensitive molecules that damage lipids, proteins, and cellular DNA, causing cell dysfunction and death [1, 2].

Numerous disorders in mammals, including infections brought on by intracellular pathogens such *Babesia* spp., have been linked to oxidative stress [3, 4]. Babesiosis is spread via ticks, particularly Rhipicephalus and Dermacentor. This sickness affects dogs all around the world. The condition is characterized by thrombocytopenia, fever, hemolytic anemia, and various organ failure [5, 6].

Hemolysis and inflammation cause an increase in ROS production, which aids in the disease's progression. Malondialdehyde (MDA) can be used to detect lipid peroxidation [7].

This study aims to evaluate MDA levels in dogs diagnosed with babesiosis, providing insights into the biochemical mechanisms of the disease.

II. Materials and Methods

Sampling

Twenty dogs were enrolled in the study, including 10 dogs diagnosed with babesiosis and 10 healthy controls. The affected dogs were medium-sized crossbreeds (15–25 kg), aged 3–8 years. Diagnosis was confirmed through microscopic examination of *Babesia* spp. in peripheral blood smears. All dogs were clinically evaluated to exclude concurrent diseases. Sterile syringes were used to venipuncture the *cephalic vein* in order to obtain blood samples. After allowing the samples to clot, serum was extracted by centrifugation for additional examination.

Laboratory Analyses

Lipid peroxidation product concentrations (MDA levels) in the serum were measured as thiobarbituric acid reactive substances (TBARS) according to the spectrophotometric method described by Costa et al. [8] with slight modifications. A 400 µl serum sample was mixed with 4 ml of a solution containing equal volumes of 15% trichloroacetic acid, 0.38% thiobarbituric acid, and 0.25 N hydrochloric acid. In addition, 40 µl of 0.2% butylated hydroxytoluene was used to prevent lipid peroxidation during heating.

The reaction mixture was vortexed and then heated in a boiling water bath for 30 minutes. After cooling in ice water for 10 minutes, the reaction mixture was centrifuged at 3000 rpm for 15 minutes. The same procedure was repeated for MDA standards and a blank test (distilled water). MDA standards were prepared by acid hydrolysis of 1,1,3,3-tetramethoxypropane (malondialdehyde-bis [dimethyl acetal]) and were diluted in the range of 0 to 2 nmol/ml.

The absorbance of the supernatant was determined at 535 nm against a blank test. TBARS concentration was read from a calibration curve prepared according to MDA standards. TBARS values in the serum were expressed in nmol/ml.

Statistical Analysis

Results were analyzed using GraphPad Prism 5. Differences between groups were assessed using an unpaired Student's t-test, with a significance threshold of $p<0.01$. The owners of the dogs agreed to blood sampling and their subsequent inclusion in the experiment. The relevant animal welfare legislative provisions were met while handling the experimental animals. Analyzed data are reported as means ± standard error of the mean (SEM)

III. Results and Discussion

The MDA levels were significantly higher in dogs with babesiosis (2.830 ± 0.4585 nmol/mL) compared to healthy controls (1.073 ± 0.726 nmol/mL, $p<0.01$), indicating heightened lipid peroxidation (Table 1).

Table 1: Comparison of Oxidative Stress Markers Between Groups

Marker	Healthy Dogs	Babesiosis Dogs
MDA (nmol/mL)	1.073	2.830
SEM	0.0726	0.4585
p	0.00136	

Note: MDA (malondialdehyde); P – significant differences if $P<0.01$ among the means by t-test; SEM – standard error of the mean.

Diseased dogs with elevated MDA levels have increased oxidative damage due to hemolysis and inflammatory responses. The increased malondialdehyde (MDA) levels in dogs with babesiosis are a result of

the severe oxidative stress of infection. Higher MDA levels have been shown in infected dogs compared to healthy ones, suggesting that Babesia parasites mainly cause oxidative damage through lipid peroxidation [10]. Babesiosis causes activation of macrophages and causes damage to lipids, proteins, and DNA, as has been shown in other parasitic diseases [4, 7]. This occurs through the production of excessive amounts of reactive oxygen species (ROS). Oxidative stress damages cells, especially red blood cells, due to the oxygen-rich environment.

An imbalance between prooxidants and antioxidants is the source of this [11]. Babesia canis is also linked to elevated levels of the oxidative stress index (OSI) and total oxidative capacity (TOC), which may indicate a systemic reaction to infection [12]. Erythrocytes and other systems may be impacted by oxidative damage, which raises the total oxidative burden [13]. Antioxidants, including polyphenols, selenium, and vitamin E, can be added to the diet of afflicted dogs to help reduce oxidative damage. A diet high in antioxidants lowers lipid peroxidation and increases enzymatic activity, claim [9, 14].

Even though oxidative stress is a major factor in babesiosis, it's vital to remember that certain dogs with elevated MDA levels may have severe clinical symptoms. This is due to the fact that a dog's reaction to oxidative stress can differ significantly based on their natural surroundings. These findings corroborate earlier research demonstrating the impact of parasite infections on oxidation. Vitamin E, selenium, and polyphenols are examples of antioxidant supplements that may help lessen the oxidative damage brought on by babesiosis.

IV. Conclusion

This study highlights how oxidative stress influences the development of canine babesiosis. Increased MDA levels suggest a role for ROS-mediated damage in disease development. Clinical outcomes may improve with the addition of antioxidants to treatment. Future studies should investigate how well different antioxidant treatment strategies control oxidative stress during babesiosis.

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References

[1] M. A. McMichael, Oxidative stress, antioxidants, and assessment of oxidative stress in dogs and cats, *Journal of the American Veterinary Medical Association*, *231(5)*, 2007, 714–720.

[2] S. Khanna, D. Pande, R. Negi, K. Karki, R. Khanna, H. Khanna, Oxidative stress induced damage in benign and malignant diseases, *Journal of Stress Physiology and Biochemistry*, (*8)*, 2012, 210–214.

[3] M. Valko, C. J. Rhodes, J. Moncol, M. Izakovic, M. Mazur, Free radicals, metals, and antioxidants in oxidative stress-induced cancer, *Chemico-Biological Interactions*, *160(1)*, 2006, 1–40.

[4] N. Hermans, P. Cos, L. Maes, T. De Bruyne, D. Vanden Berghe, A. J. Vlietinck, L. Pieters, Challenges in antioxidant research, *Current Medicinal Chemistry*, *14(4)*, 2007, 417–430.

[5] V. Matijatko, M. Torti, T. P. Schetters, Canine babesiosis in Europe: how many diseases?, *Trends in parasitology*, *28 (3)*, 2012, 99-105.

[6] P. J. Irwin, Canine babesiosis: from molecular insights to clinical advancements, *Parasites and vectors*, *(2)*, 2009, S4.

[7] B. Halliwell, Oxidative stress, *British journal of pharmacology*, *(120)*, 1997, 378.

[8] C. M. Costa, R. C. C. Santos, E. S. Lima, A simple automated procedure for thiol measurement in human serum samples, *Jornal brasileiro de patologia e medicina laboratorial*, *42(5)*, 2006, 345-350.

[9] D. M. Hagen, J. L. Ekena, B. M. Geesaman, K. R. Viviano, Antioxidant supplementation during illness in dogs: effect on oxidative stress and outcome, an exploratory study, *Journal of Small Animal Practice*, *6 (9)*, 2019, 543-550.

[10] M. Crnogaj, R. Petlevski, V. Mrljak, I. Kiš, M. Torti, N. Kučer, V. Matijatko, I. Sacer, I. Stokovic, Malondialdehyde levels in serum of dogs infected with Babesia canis, *Veterinarni Medicina, 55(4)*, 2010, 163–171.

[11] S. M. Razavi, M. S. Soltan, K. Abbasian, A. Karami, S. Nazifi, Host oxidative stress in piroplasmosis: A review in domestic animals, *Veterinary Parasitology*, *322*, 2023, 110011.

[12] M. O. Tarhan, Y. Deger, B. Oguz, U. Ozdek, Investigation of Thiol-Disulphide Homeostasis, Total Oxidant-Antioxidant, and Ischaemia-Modified Albumin Levels in Dogs with Babesiosis, *Alexandria Journal of Veterinary Sciences, 77(1)*, 2023, 21–21.

[13] I. Rubic, R. Burchmore, S. Weidt, C. Regnault, J. Kuleš, R. Barić Rafaj, T. Mašek, A. Horvatić, M. Crnogaj, P. D. Eckersall, P. Novak, V. Mrljak, Multi-platform strategies and metabolomics approaches for the investigation of comprehensive metabolite profile in dogs with Babesia canis infection, *International Journal of Molecular Sciences, 23(3)*, 2022, 1575.

[14] M. G. Hayek, Editorial: Potential for Control of Oxidative Injury by Administration of Antioxidants in Veterinary Medicine, *Journal of Veterinary Internal Medicine, (19)*, 2005, 301-302