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Catalase and Glutathione Peroxidase in Dogs Naturally Infected by *Leishmania infantum*

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ABSTRACT

***Background*:** Canine leishmaniasis (CanL) is caused by an obligatory intracellular parasite of *Leishmania* genus that affects organs and tissues. Several studies evaluate the role of reactive oxygen species (ROS) in the pathogenesis of many diseases. The overproduction of ROS on infectious diseases can induce an imbalance between oxidants and antioxidants at cellular or systemic level. Thus, the aim of this study was to evaluate the activity of antioxidant enzymes in CanL.
***Materials, Methods & Results*:** Females (n = 17) and males (n = 10), at different ages and with different weight, were selected for this study. Dogs were divided into two groups according classical clinical signs and sorological test to CanL. Animals were considered infected based on indirect immunofluorescent assay and ELISA titration ≥ 1:40. Group B (n = 15) composed by positive dogs to CanL from Zoonosis Control Center of Fortaleza (Ceará, Brazil) and group A (n = 12) was composed by dogs from private kennel that were serologically negative to *L. infantum* and had absence of clinical signs to CanL. Blood sample were collected for evaluation of hematological and biochemical parameters and glutathione peroxidase (GPx) and catalase (CAT) enzymatic activity. Data were analyzed by Student's t-test and Pearson correlation coefficient ($P < 0.05$). Total proteins (TP, mg/dL) and alkaline phosphatase (ALP, U/L) were increased ($P < 0.05$) on group B (8.2 ± 1.2; 165.4 ± 46.4) when compared to group A (6.5 ± 1.1; 109.1 ± 38.3), respectively. Hemoglobin (Hb; g/dL) and hematocrit (Hct; %) were decreased ($P < 0.05$) on Group B (14.7 ± 1.8; 48.2 ± 5.7) when compared to group A (16.5 ± 1.3; 52.1 ± 2.4), respectively. Group B presented CAT (U/g Hb) and GPx (mU/mg Hb) lower (189.4 ± 90.4; 3,609.6 ± 1,569.1) than group A (326.6 ± 104.5; 5,055.6 ±1,569.1), respectively ($P < 0.001$). Positive correlation was observed between RBC and CAT; however, it was not significant.
Discussion: Organisms require a good defense system in order to revert the overproduction of free radicals and consequently the injuries caused by them. This is possible through the production of antioxidant agents, which act on oxidative prevention and on tissue and cellular regeneration, by taking the reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) enzymes in the frontline. Erythrocyte changes promoted by CanL suggest possible correlation between anemia and the appearance of clinical signs, which in many cases is not seen. Erythrocytes contain SOD, CAT and GPx enzymes, thus, changes in these cells will reflect on the activity of these enzymes. In our results only CAT showed positive correlation with erythrocyte count, however it was not significant. GPx activity was lower ($P < 0.001$) in infected dogs than control group. This result agrees with another study, which showed a decrease in GPx levels in CanL, although it was not significant. However, it was found a positive correlation ($P < 0.001$) between erythrocytes and GPx activity and between hemoglobin and GPx activity in animals with leishmaniasis. These results suggest that the reduction in detoxification activity can be related to the decrease in erythrocyte count and that the GPx activity depends on the control mechanism of the antioxidant system in CanL. Furthermore, this result could be associated with decrease of blood cell count in animals with CanL, once GPx is an erythrocyte enzyme, which plays an important role in hemoglobin protection against oxidative damage. This study was carried out in naturally infected dogs with *L. infantum*. In conclusion, CAT and GPx activities are relate to oxidative stress induced by *L. infantum* infection and can be used as biomarkers on CanL.

Keywords: canine leishmaniasis, antioxidants enzymes, oxidative stress, biomarkers.

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INTRODUCTION

Canine leishmaniasis (CanL) is an anthropozoonosis caused by a protozoan of genus *Leishmania*. It is transmitted through the bite of insects Diptera, family Psychodidae, genus *Phlebotomus* and *Lutzomyia* [3]. The different *Leishmania* spp. are responsible for a wide spectrum of disorders that affect mammals, as men and animals, which may act as hosts and reservoirs [27,29].

CanL is a systemic disease that could affect different organs and tissues, promoting clinical-pathological changes, which characterize different clinical presentations [30]. These clinical features depend on the immune response of the dog and the participation of the immune system at *Leishmania* control is critical for progression or resolution of the disease [9,14,33].

Recent studies have suggested the involvement of reactive oxygen species (ROS) on the pathogenesis of various infectious and parasitic diseases in dogs [10,13,18,20,31,34] including CanL [5,7,8,16]. ROS overproduction can induce an imbalance between oxidants and antioxidants at cellular or systemic level, leading to the establishment of oxidative stress. This process can result in the oxidation of biomolecules with consequent loss of their biological functions [6] or a homeostatic imbalance that is manifested by the modification of macromolecules, cell death (apoptosis or necrosis), as well as structural tissue damage [11].

Once there is involvement of oxidative stress in physiological and pathological mechanisms, it is necessary to know the role of antioxidants in CanL. Thus, the aim of this study was to evaluate the antioxidant enzymatic activity in dogs naturally infected by *L. infantum*.

MATERIALS AND METHODS

Animals

Twenty-seven mongrel dogs, females (n = 17) and males (n = 10), at different ages and with different weight, were selected for this study. Test group was composed by animals from Zoonosis Control Center of Fortaleza (Ceará, Brazil) that presented positive serology to *L. infantum* and control group was composed by dogs from private kennel that were serologically negative to *L. infantum*. The Ethics Committee for Animal Use of the State University of Ceará (CEUA/UECE) approved experimental protocol, under protocol number 11516675-0/65.

Serological tests

The Enzyme-linked immunosorbent assay (EIE)[1] and Indirect Immunofluorescence (IFI)[1] were used to analyze the seroreactivity of each sample [23]. For these assays, the cutoff points were considered a titer higher than 1:40.

Experimental groups

The animals were divided into two groups. Group A (n = 12) was composed by dogs that were negative for *L. chagasi* infection and Group B (n = 15) by positive dogs. The animals that were serologically positive for the disease, also presented more than three clinical signs strongly associated with CanL, such as cachexia, onychogryphosis, hepatosplenomegaly, lymphadenopathy, alopecia, skin disorders and others symptoms [15]. Control dogs were serologically negative and did not present clinical signs.

Hematological and biochemical analysis

From each dog, 8 mL of blood were collected through jugular venipuncture and the blood was placed in tubes with EDTA (4 mL) and in tubes without anticoagulant (4 mL). Samples blood were subject to an automated blood analyzer (BC-2800 Vet)[2] for complete blood count. Hematological parameters were evaluated for white blood cells (WBC, 103/dL), including total leukocytes and differential leukocytes, neutrophils (Neu), eosinophils (Eos), monocytes (Mon), basophils (Bas), and lymphocytes (Lym); red blood cells: erythrocytes (RBC, 106/dL), hemoglobin (Hb, g/dL), hematocrit (Hct, %) and total platelets (Plt, $x10^3$/dL). Levels of urea (U, mg/dL), creatinine (Crea, mg/dL), total proteins (TP, g/dL), and the enzymatic activity of glutamic oxaloacetic transaminase (GOT, U/L), glutamic pyruvic transaminase (GPT, U/L) and alkaline phosphatase (ALP, U/L) were determined by an automated system[3] using specific commercial kits[3].

Results of hematological and biochemical parameters were analyzed in relation to reference values for canine species [17].

Antioxidants enzymes activity

Catalase (CAT) activity [1] was measured after isolation and lysis of RBCs while glutathione peroxidase (GPx) activity was determined on whole blood, using commercial kits[4]. CAT was expressed as U/g Hb and GPx as mU/mg Hb.

Statistical analysis

All values were expressed as the mean ± SD. Data were assessed by comparing the results of the treatment groups with those of the control using Student's t-test. Pearson correlation coefficient was used. $P < 0.05$ was considered significant.

RESULTS

The hematological and biochemical evaluation data are given in Tables 1 and 2. There was no significant difference among the parameters expressed in Table 1 between groups, and all data found within the reference values for dogs. However, the concentrations of TP and ALP were higher in Group B. These data suggest an increase on the production of immunoglobulin associated with the immune response to *L. infantum*. The high levels of ALP when associated with an increase of gamma-glutamyl transferase (not determined) suggest liver damage.

As the antioxidant enzymatic activity was performed in erythrocytes, the red blood cells parameters were placed together with results of oxidative stress evaluation (Table 2). RBC count ($P < 0.001$), Hct concentration ($P < 0.05$) and Hb content ($P < 0.01$) were decreased in dogs with CanL (group B), when compared to the control group (group A).

CAT and GPx levels were lower in the animals of group B and these values were significant ($P < 0.001$). In an attempt to show a possible correlation between hematological parameters and oxidative stress markers, a positive correlation between RBC and CAT activity were verified; however, this data was not significant.

Table 1. Leukocytes and biochemical parameters in healthy and naturally infected dogs by *L. infantum*.

Parameter	Group A (Control, n = 12)	Group B (CanL, n = 15)
Leukocytes (/dL)	12,934 ± 1,777	12,473 ± 2,429
Neutrophils (%)	60.5 ± 17.7	68.0 ± 5.5
Lymphocytes (%)	35.8 ± 19.9	30.2 ± 5.1
Monocytes (%)	3.3 ± 2.9	1.7 ± 1.4
Platelets (x10^3)	298 ± 82	254 ± 213
Urea (mg/dL)	33.3 ± 10.0	16.857 ± 6.21
Creatinine (mg/dL)	1.3 ± 0.2	1.0 ± 0.8
Total protein (g/dL)	6.5 ± 1.1[a]	8.2 ± 1.2[b]
GOT (U/L)	30.1 ±13.4	24.1 ± 11.7
GPT (U/L)	27.1 ± 13.5	42.8 ± 12.9
ALP (U/L)	109.1 ± 38.3[a]	165.4 ± 46.4[b]

Small letters in the same line means a significant difference ($P < 0.01$).

Table 2. Red Blood Cells (RBC) and antioxidant enzymatic levels on blood sample at healthy and naturally infected dogs by *L. infantum*.

Parameter	Group A (Control, n=12)	Group B (CanL, n=15)	*P*
RBC (x10^6/dL)	7.0 ± 0.6	5.6 ± 0.4	$P < 0.001$
Hct (%)	52.1 ± 2.4	48.2 ± 5.7	$P < 0.05$
Hb (g/dL)	16.5 ± 1.3	14.7 ± 1.8	$P < 0.01$
CAT (U/g Hb)	326.6 ± 104.5	189.4 ± 90.4	$P < 0.001$
GPx (mU/mg Hb)	5,055.6 ± 1,569.1	3,609.6 ± 1,569.1	$P < 0.001$

DISCUSSION

Oxidative stress is produced physiologically in healthy individuals, but compensation mechanisms are quickly activated for the return of homeostasis. However, most sick individuals show an unbalance in redox mechanisms, promoting the occurrence of an intense oxidative stress [29]. Thus, organisms require a good defense system in order to revert the overproduction of free radicals and consequently the injuries caused by them. This is possible through the production of antioxidant agents, which act on oxidative prevention and on tissue and cellular regeneration, taking the reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) enzymes in the frontline [4,28].

Canine leishmaniasis (CanL) is caused by an obligatory intracellular parasite characterized by chronical evolution with systemic involvement of host several tissues and organs [25]. The clinical signs of CanL have been associated to immune changes that involve cells of the mononuclear phagocytic system, different populations of effector lymphocytes Th1, Th2, Treg, characterized by specific cytokine secretion of cellular subpopulations and the production of specific antibodies of different subclasses of immunoglobulins [2,9,14].

The unbalance of oxidant and antioxidant agents is seen on several pathological situations, including the infectious and parasitic diseases. In our study, we emphasized the oxidative stress markers through the evaluation of CAT and GPx (Table 2) enzymatic activity, which were detected in erythrocytes. CAT is a cytoplasmic heme protein that converts hydrogen peroxide (H_2O_2) in oxygen and water [22]. CAT enzyme activity is present in all tissues, though its function is higher in erythrocytes, liver, kidneys, fat, and lower in nervous tissue [35]. The high production of H_2O_2 must be managed by the organism through the activity of antioxidant enzymes GPx and CAT, which could lead to a high catalytic demand of these enzymes. GPx is an enzyme who catalyzes the H_2O_2 reduction using glutathione as substrate along with other organic hydroperoxides [4].

Concerning the hematologic parameters, in this study, the erythrocyte count, hematocrit and hemoglobin levels of naturally infected dogs with *L. infantum* were lower ($P < 0.05$) when compared to control group. It has been reported that anemia is one of the most common findings in CanL, affecting about 60% of infected animals, and in some situations, it is characterized as severe in symptomatic dogs [15]. The erythrocyte changes observed in dogs with leishmaniasis (Group B) suggest a possible correlation between anemia and the appearance of clinical signs, which in many cases is not seen [30]. Erythrocytes contains large amounts of SOD, CAT and GPx enzymes, thus, changes in these cells will reflect on the activity of these enzymes. In our study, the antioxidants status was determined through CAT and GPx.

GPx activity was lower ($P < 0.001$) in infected dogs than control group. This result agrees with the study of Britti *et al*. [8], which showed a decrease in GPx levels in CanL, although it was not significant. However, these authors found a positive correlation ($P < 0.01$) between erythrocytes and GPx activity and between hemoglobin and GPx activity in animals with CanL. These results suggest that the reduction in detoxification activity can be related to the decrease in erythrocyte count and that the GPx activity depends on the control mechanism of the antioxidant system in CanL. Furthermore, this result could be associated with decrease on RBC count at CanL, thus GPx is an erythrocyte enzyme that plays an important role in hemoglobin protection against oxidative damage [24]. Decrease on GPx activity have been demonstrated in animals with scabiosis [34].

In relation to lipid peroxidation in CanL it was found high level of MDA and that the non-enzymatic antioxidants like ascorbic acid levels, β-carotene and ceruloplasmine were lower in sick animals [7,16]. Also, it was observed that single and co-infections by *L. infantum*, *E. canis* and *B. vogeli* in dogs cause an increase in the levels of nitric oxide (NO), advanced oxidation protein products (AOPP) and antioxidants as ferric reducing antioxidant power (FRAP) [5,16]. Our studies revealed high levels of ALP, which can be associated to liver damage promoted by lipid peroxidation. However, gamma-glutamyl transferase was not determined and TGO and TGP did not change significantly in this study.

Another important antioxidant enzyme identified in the present study was catalase (CAT). Animals with CanL showed lower ($P < 0.001$) CAT activity compared to control group, and CAT showed positive correlation with the global erythrocyte count, however it was not significant. Other authors observed an increase in CAT activity in dogs with parvovirus infection [28] and babesiosis [8].

At CanL it was verified that the intensity of oxidative stress was dependent on the disease stage and it was associated with increased induced production of superoxide and apoptosis [8,11]. Furthermore, symptomatic dogs for CanL had a decrease on total antioxidant status (TAS) that supplied specific effects on the antioxidant defense mechanisms [16].

CONCLUSION

Based on our results, we can conclude that CAT and GPx activities are relate to oxidative stress induced by *L. infantum* infection and can be used as biomarkers on CanL. This study is relevant because it was carried out in dogs naturally infected with *L. infantum*.

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***Ethical approval*.** The present study was approved by the animal experimentation committee, CEUA/UECE, under protocol 11516675-0/65.

***Declaration of interest*.** The authors declare that there is no conflict of interest.

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