Nitric Oxide, Protein Oxidation and Total Antioxidant Levels in Serum of Dogs Naturally Infected by *Ehrlichia canis*, *Leishmania infantum* and *Babesia vogeli*

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ABSTRACT

**Background:** *Ehrlichia canis* and *Babesia vogeli* comprise a group of globally distributed pathogens transmitted by ticks. *Leishmania infantum* is transmitted by *Lutzomyia longipalpis*, etiological agent of leishmaniasis. The pathogens affect the animals; and can also affect the human. An imbalance between oxidants and antioxidants compounds causes an increase in free radicals, and other reactive oxygen species (ROS) has an important role in tissue damage in a variety of pathological processes, as parasitic disease. The objective of this study was to evaluate the nitric oxide levels, protein oxidation and total antioxidant status in serum of dogs infected by *L. infantum*, *E. canis* and *B. vogeli*.

**Materials, Methods & Results:** Blood samples from dogs originating from the city of Campo Grande, Mato Grosso do Sul state, Brazil were collected to research hemoparasites by polymerase chain reaction (PCR) using specific primers for each parasite. Serum samples obtained from 54 dogs with single and co-infection were used this study: *L. infantum* (n = 19), *E. canis* (n = 8), *B. vogeli* (n = 5), *L. infantum* and *E. canis* (n = 12), *L. infantum* and *B. vogeli* (n = 4), and *E. canis* and *B. vogeli* (n = 6), as well as 17 normal controls (uninfected). Samples were stored at -80°C for further evaluation of NOx, AOPP, and FRAP levels using Cobas Mira automated analyzer, and results were expressed as µmol/L. NOx, AOPP and FRAP levels were increased (P < 0.01) in dogs infected by parasites when compared to uninfected animals. Levels of NO- and AOPP in dogs naturally infected by *Babesia vogeli* and *Ehrlichia canis*, respectively, did not differ from the control group (uninfected). The higher NOx levels were observed in the serum of dogs with co-infection by *L. infantum* and *E. canis*, and *L. infantum* and *B. vogeli* (P < 0.01). Altogether, our results indicate that dogs naturally infected by *L. infantum*, *E. canis* and *B. vogeli* developed a state of redox imbalance.

**Discussion:** Oxidative stress in dogs infected by *L. infantum*, *Ehrlichia canis*, and *B. vogeli*, single or co-infected were verified this study. As a result, naturally infected dogs sampled in the present study showed augmented levels of ROS, as well as in serum samples of dogs infected by *Babesia gibsoni*, *Leishmania spp.*, *Hepatozoon canis*, and *Trypanosoma evansi*. Nitric oxide increased (detected by high NOx levels) can be a marker of oxidative stress, as well as an important mediator of inflammatory response. In experimental conditions, an increased level of NOx in dogs infected with *E. canis* was observed, and researchers say this increase was directly related to the pathogenesis of the disease. The imbalance of oxidants and antioxidants is present in various animal species in many pathological situations including parasitic diseases, as in dogs infected by hemoparasites showed increased oxidative markers (NO- and AOPP) associated with increased antioxidant levels (FRAP), which features a status of oxidative stress. The increase of FRAP is interpreted as the elevation of total antioxidant levels, i.e. enzymatic and non-enzymatic. The increased antioxidant is good to the host, it has the function of neutralizing the oxidizing or reducing that when high levels are toxic to cells and tissues. Therefore, we suggest that the infections caused by these hemoparasites cause oxidative stress and this process may participate in disease pathology.

**Keywords:** babesiosis, ehrlichiosis, leishmaniasis, canine, oxidative stress.
INTRODUCTION

Ehrlichiosis, babesiosis, and leishmaniasis are considered some of the major impediments in health and productive performance of many animals [10,22]. *Rhipicephalus sanguineus* is the main vector of *Ehrlichia canis* and *Babesia vogeli* among dogs [10,23,27], as well as *Lutzomyia longipalpis* is the vector of *Leishmania* spp. Visceral Leishmaniasis (VL) is a parasitic disease of humans and other mammals, caused by protozoa of the *Leishmania donovani* complex [1]. *E. canis* is a pleomorphic gram-negative obligate bacterium, and the causative agent of canine monocytic ehrlichiosis [15,18,27]. Babesiosis is a clinically noteworthy and well-known hemoprotozoan parasite of dogs and other domestic animals, causing severe clinical sings [9]. These infections cause similar clinical alterations such as anemia, but they may also be asymptomatic in dogs.

Oxidative stress results from an imbalance between oxidants and antioxidants compounds in favor of the excessive generation of free radicals [13], and other reactive oxygen species (ROS) play an important role in tissue damage in a variety of pathological processes [19]. Nitric oxide (NO-) reacts with biological molecules, such as superoxide anion and oxyhemoglobin to form nitrites and nitrates [25]. The mensuration of NO- levels may be a parameter to evaluate oxidative stress status [3]. Advanced oxidation protein products (AOPP) is a biomarker used to assess oxidative stress [2], since it measures the levels of protein oxidation. Therefore, the aim of this study was to evaluate the nitric oxide levels, protein oxidation and total antioxidant status in serum of dogs infected by *L. infantum*, *E. canis* and *B. vogeli* (single and co-infected).

MATERIALS AND METHODS

**Serum sampling**

Blood samples from dogs originating from the city of Campo Grande, (Mato Grosso do Sul state, Brazil) were collected to research hemoparasites. Molecular diagnosis was performed by polymerase chain reaction (PCR) using specific primers for each parasite [24]. On the day of blood collection, a volume of 3 mL of whole blood was stored in tubes without anticoagulant. The serum to biochemical analyzes was prepared by centrifugation of whole blood at 3500 g for 10 min.

Methodology

Samples obtained from 54 dogs with single and co-infection (*L. infantum* (n = 19), *E. canis* (n = 8), *B. vogeli* (n = 5), *L. infantum* and *E. canis* (n = 12), *L. infantum* and *B. vogeli* (n = 4), *E. canis* and *B. vogeli* (n = 6)] and 17 normal controls (uninfected) were stored at -80ºC for further evaluation of NOx, AOPP, and FRAP levels. Measurement of these variables was performed using Cobas Mira¹ automated analyzer, and results were expressed as µmol/L. Nitric oxide levels in serum of infected and uninfected dogs were evaluated indirectly, by nitrite/nitrate (NOx) quantification according to the technique described by Tatsch et al. [25], using 50 µL of sample. Protein oxidation was assessed through the measurement of AOPP (advanced oxidation protein products) concentrations by a method previously described by Hanasand et al. [14]. Levels of ferric reducing antioxidant power (FRAP) was measured according to the technique described by Benzie and Strain [4].

**Statistical analysis**

Data were subjected to normality test and results that showed abnormal distribution (FRAP levels) were log transformed. Normal data were subjected to Tukey test. Values with probability (P) less than 5 % were considered statistically different. Data were presented as mean values ± standard error of the mean (SEM).

RESULTS

NOx, AOPP and FRAP levels in serum are shown in Table 1. Compared to control, NOx levels were increased in serum of dogs infected by *L. infantum*, *E. canis*, *L. infantum* and *E. canis*, *L. infantum* and *B. vogeli*, and *E. canis* and *B. vogeli*. It may be noted that co-infections showed higher levels of NOx compared to single infections (P < 0.01), except in dogs co-infected by *E. canis* and *B. vogeli*. AOPP levels showed higher values in serum of all infected groups when compared to uninfected dogs, except for dogs presenting single infection by *E. canis*. FRAP levels showed increased values in serum samples of all infected groups when compared to uninfected dogs.
Table 1. Nitrite/nitrate (NOx), advanced oxidation protein products (AOPP), and ferric reducing antioxidant power (FRAP) levels in serum of dogs infected by Leishmania infantum, Ehrlichia canis and Babesia vogeli.

<table>
<thead>
<tr>
<th>Group</th>
<th>NOx (µmol/L)</th>
<th>AOPP (µmol/L)</th>
<th>FRAP (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected (n = 17)</td>
<td>55.5 ± 4.4c</td>
<td>5.5 ± 0.3c</td>
<td>766.6 ± 87.8c</td>
</tr>
<tr>
<td>L. infantum (n = 19)</td>
<td>97.8 ± 5.9b</td>
<td>14.3 ± 2.0ab</td>
<td>1773.2 ± 130.9a</td>
</tr>
<tr>
<td>E. canis (n = 8)</td>
<td>82.9 ± 12.6b</td>
<td>7.1 ± 0.9bc</td>
<td>1417.2 ± 348.1ab</td>
</tr>
<tr>
<td>B. vogeli (n = 5)</td>
<td>51.1 ± 9.3c</td>
<td>17.8 ± 1.6ab</td>
<td>1894.2 ± 510.3a</td>
</tr>
<tr>
<td>L. infantum and E. canis (n = 12)</td>
<td>174.6 ± 26.5a</td>
<td>19.2 ± 3.0a</td>
<td>1898.9 ± 245.6a</td>
</tr>
<tr>
<td>L. infantum and B. vogeli (n = 4)</td>
<td>224.5 ± 76.5a</td>
<td>15.4 ± 5.0b</td>
<td>1676.5 ± 308.1a</td>
</tr>
<tr>
<td>E. canis and B. vogeli (n = 6)</td>
<td>94.4 ± 9.3b</td>
<td>10.9 ± 0.8b</td>
<td>1052.3 ± 232.5b</td>
</tr>
</tbody>
</table>

P <0.01 <0.001 <0.01

DISCUSSION

In this study it was verified the occurrence of oxidative stress in dogs infected by L. infantum, Ehrlichia canis, and B. vogeli, single or co-infected. Therefore, naturally infected dogs sampled in the present study showed augmented levels of ROS, which corroborate to previous works which evaluated serum samples of dogs infected by Babesia gibsoni [17], Leishmania spp. [5], Hepatozoon canis [16] and Trypanosoma evansi [28].

All infected dogs (except single infection by B. vogeli) showed increased serum levels of nitric oxide resembling to hepatozoonosis [16] and canine rangeliosis [20]. In vitro studies showed that NO· has the ability to kill parasites like T. cruzi [26]. However, NO· increase can be a marker of oxidative stress, as well as an important mediator of inflammatory response [8]. Large amounts of NO· for relatively long periods may produce some undesired effects, such as production of toxic compounds to the cell or tissue and oxidative reactions, such as lipid and protein damage [11]. In experimental conditions, an increased level of NOx in dogs infected with E. canis was observed, and according to the authors, this increase can be toxic, and may contribute to the pathogenesis of canine ehrlichiosis, aggravating the clinical symptoms [7].

The imbalance of oxidants and antioxidants is present in various animal species in many pathological situations including parasitic diseases [21]. Dogs infected by hemoparasites showed increased oxidative markers (NO- and AOPP) associated with increased antioxidant levels (FRAP), which features a status of oxidative stress, as described in other diseases such as those caused by Leishmania chagasi [5], Babesia canis [6] and T. evansi [8]. High AOPP indicates a situation of protein oxidation caused by cellular lesions, as observed in canine rangeliosis [12] and ehrlichiosis [7].

Based on these results, it was concluded that infections caused by L. infantum, E. canis and B. vogeli (single and co-infections) in dogs cause an increase in the levels of nitric oxide, oxidized protein and antioxidants. In summary, we suggest that the infections caused by these hemoparasites cause oxidative stress and this process may participate in disease pathology. An important finding of this study was that changes previously described in experimental infections with E. canis [25] showed similar results to those found in naturally infected dogs.

REFERENCES


